9-11-2006

Provisional Peer Reviewed Toxicity Values for

Iron and Compounds
(CASRN 7439-89-6)

Derivation of Subchronic and Chronic Oral RfDs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISONAL PEER REVIEWED TOXICITY INFORMATION FOR IRON (CASRN 7439-89-6) AND COMPOUNDS
Derivation of Subchronic and Chronic Oral RfDs

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI

INTRODUCTION

A reference dose (RfD) for iron is not available on the Integrated Risk Information System (IRIS) (U.S. EPA, 2006) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2005). The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997) reported that data regarding iron were inadequate for quantitative risk assessment. The Chemical Assessment and Related Activities (CARA) list (1991, 1994) includes a Health Effects Assessment (HEA) for Iron and Compounds (U.S. EPA, 1984) that found no reliable quantitative oral toxicity data. Iron has not been the subject of a toxicological review by the Agency for Toxic Substances Disease Registry (ATSDR) (2005) or the World Health Organization (WHO) (2005). Monographs by the International Agency for Research on Cancer (IARC) (1972, 1987), toxicity reviews by Jacobs (1977), Bothwell et al. (1979), Lauffer (1991) and Grimsley (2001), a review on dietary iron by the National Academy of Sciences (NAS) (2001), and the National Toxicology Program (NTP) (2001, 2005) management status report and chemical repository summary were consulted for relevant information. The NAS (2001) derived a Tolerable Upper Intake (TUI) level of 45 mg iron/day. The TUI is based on a minimal LOAEL of 70 mg/day (60 mg iron as ferrous fumerate plus 11 mg/day of dietary iron) identified by Frykman et al. (1994) for gastrointestinal effects and an uncertainty factor of 1.5 for use of a minimal LOAEL; a higher

uncertainty factor was not used since the nature of the observed gastrointestinal effects was considered to be self-limiting. The U.S. Food and Drug Administration (FDA) promulgated a Rule in 1997 for labeling of iron-containing dietary supplements for the prevention of accidental poisoning in children (U.S. FDA, 1997). The Rule, as modified in 2003, does not contain specific exposure limits (U.S. FDA, 2003). In general, the FDA follows the NAS guidance on exposure limits for toxicity of essential elements, such as iron. Previous literature searches were conducted through September, 2001 as follows: TOXLINE (oral and inhalation toxicity and cancer from 1983 - September, 2001); CANCERLIT (1990 - September, 2001); MEDLINE (1991 - September, 2001); TSCATS, RTECS, DART/ETICBACK, EMIC/EMICBACK, HSDB, GENETOX, and CCRIS. Update literature searches were performed in October, 2005 in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents.

REVIEW OF PERTINENT LITERATURE

Iron is an essential element and deriving a risk assessment value for such chemicals poses a special problem in that the dose-adversity curve is "U-shaped". Thus, the risk value must be protective against deficiency as well as toxicity. The NAS (2001) has established guidelines for iron intake that take into account physiological differences during different life stages. For non-breast-fed infants aged 0-6 months, the NAS (2001) established a daily adequate intake (AI) for iron of 0.27 mg/day (0.04 mg/kg-day for infants 2-6 months old) based on the daily amount of iron secreted in human milk; breast-fed infants typically receive only 0.15 to 0.3 mg Fe/day. The NAS (2001) Dietary Reference Intakes (DRIs) for children are as follows: 11 mg/day (1.2 mg/kg-day) for infants between the ages of 7 and 12 months, 7 mg/day (0.54 mg/kg-day) for children aged 1-3 years, 10 mg/day (0.45 mg/kg-day) for ages 4-8 years, 8 mg/day (0.2 mg/kg-day) for ages 9-13 years and 11 mg/day (0.17 mg/kg-day) for boys and 15 mg/day (0.26 mg/kg-day) for girls aged 14-18 years. The DRI for men aged 19 years and above is 8 mg/day (0.11 mg/kg-day). The DRI for non-pregnant women is 18 mg/day (0.29 mg/kg-day) for ages between 19 and 50 years and 8 mg/day (0.13 mg/kg-day) for ages 51 years and older. The DRI for pregnant women is 27 mg/day (0.37 mg/kg-day for those aged 14-18 years and 0.35 mg/kg-day for those aged 19-50 years). The DRI during lactation is 10 mg/day (0.18 mg/kg-day) for women aged 14-18 years and 9 mg/day (0.15 mg/kg-day) for women aged 19-50 years.

According to the Centers for Disease Control and Prevention (CDC, 1998; CDC, 2005), iron deficiency is one of the most common known forms of nutritional deficiency. Its prevalence is highest among young children and women of childbearing age, particularly pregnant women. In children, iron deficiency causes developmental delays and behavioral disturbances, and in pregnant women, it increases the risk for a preterm delivery and delivering a low-birthweight baby. Young children are at great risk of iron deficiency because of rapid growth and increased iron requirements. Iron deficiency can occur due to lack of iron in the diet. If this continues, anemia results. Anemia is a manifestation of iron deficiency when it is relatively severe. Iron deficiency anemia significantly impairs mental and psychomotor development in infants and children. Although iron deficiency can be reversed with treatment, the reversibility of the mental and psychomotor impairment is not yet clearly understood. Thus, prevention and treatment need to be emphasized more than detection. In addition, iron deficiency increases a child's

susceptibility to lead toxicity. Lead replaces iron in the absorptive pathway when iron is unavailable.

In humans and other animals, levels in the body are regulated primarily through changes in the amount of iron absorbed by the gastrointestinal mucosa. The absorption of dietary iron is influenced by body stores, by the amount and chemical nature of iron in ingested food and by a variety of dietary factors that increase or decrease the availability of iron for absorption (Hillman, 2001; Santi and Masters, 2001). Iron contained in meat protein (hemoglobin and myoglobin) is absorbed intact without first being broken down to elemental iron. Non-heme iron must first be reduced to ferrous iron (Fe^{2+}) before it can be absorbed. Ferrous iron is transported across intestinal mucosal cells by active transport with the rate of transport inversely related to body iron stores. Depending upon the iron status of the body, iron is stored bound to ferritin within mucosal cells and macrophages in the liver, spleen and bone, or is transported in the plasma bound to transferrin. Serum levels of ferritin and transferrin, along with several red blood cell parameters, can be used clinically to evaluate iron balance. Although iron absorption is regulated, excessive accumulation of iron in the body resulting from chronic ingestion of high levels of iron cannot be prevented by intestinal regulation and humans do not have a mechanism to increase excretion of absorbed iron in response to elevated body levels (NAS, 1989, 2001).

Human Studies

Acute Exposure

Information on acute oral toxic doses of iron in humans is available from numerous case reports of ingestion by children, but values vary because it is difficult to obtain accurate estimates of the amount taken in most overdose situations. Reviews of these case reports indicate that doses in the range of 200-300 mg iron/kg are generally considered lethal (Arena, 1970; Krenzelok and Hoff, 1979; NRC, 1979; Engle et al., 1987; Mann et al., 1989; Klein-Schwartz et al., 1990).

Therapeutic Studies

Ferrous salts are administered orally for the therapeutic treatment of iron deficiency. The oral absorption of ferrous iron supplements is considered to be essentially the same for all ferrous salts (e.g., sulfate, fumarate, succinate and gluconate) and is approximately three times greater than that of ferric (Fe^{3+}) salts (Hillman, 2001); thus, ferric iron is not used therapeutically. Constipation and other gastrointestinal effects, including nausea, vomiting, diarrhea and gastrointestinal pain are commonly associated with administration of oral ferrous salt supplements (Hillman, 2001; Santi and Masters, 2001). Severity of effects is variable, ranging from mild to severe, and depends upon dose and individual susceptibility. The onset of symptoms typically occurs at the initiation of treatment and continues throughout the duration of treatment. Although there is no indication that the severity of gastrointestinal effects varies over the course of treatment, severity is decreased in some patients when iron supplements are administered with food (Hillman, 2001; Santi and Masters, 2001). For most patients, iron deficiency is reversed within six months of treatment, thus limiting the duration of exposure.

The mechanism of iron-induced gastrointestinal toxicity is not established, although it is postulated that adverse effects are due to irritant effects of the free iron ion on the gastric muscosa (Liguori, 1993). The role of absorbed iron in the development of gastrointestinal adverse effects is unknown. The adverse effects of exposure to oral iron supplements has been investigated in several studies (Blot et al., 1981; Brock et al., 1985; Coplin et al., 1991; Fryklman et al., 1994; Hallberg et al., 1966; Liguori, 1993).

Frykman et al. (1994) evaluated the adverse effects of daily oral therapy with iron fumarate in a double-blind, crossover, placebo-controlled study in Swedish male [n=25; mean age 45 years (range 40-52)] and female [n=23; mean age 41 years (range 34-45)] adult blood donors. Study subjects were administered 60 mg elemental iron as a daily dose of iron fumarate for one month, with each study subject serving as their own placebo control. Compared to the placebo treatment period, the percentage of subjects reporting constipation (placebo 20%, ferrous fumarate 35%, $p<0.05$) and total gastrointestinal symptoms (nausea, obstipation, gastric pain and diarrhea (placebo 14%, ferrous fumarate 25%, $p<0.01$) was significantly increased during ferrous fumarate treatment. Although the severity of gastrointestinal effects was graded as minor in most study subjects, four subjects withdrew from the study due to severe gastrointestinal symptoms associated with iron fumarate. In a matched group of 49 adults taking a daily combination supplement of porcine-derived heme-iron and iron fumarate containing a total daily supplement of 18 mg iron/per day, the frequency of gastrointestinal symptoms was not increased compared to placebo. No differences in therapeutic efficacy, as measured by serum ferritin and hemoglobin levels, were observed between the non-heme iron and heme-iron treatment groups.

Adverse effects of four oral iron preparations were evaluated in 1496 male and female adult blood donors in a series of double-blind, placebo controlled trials (Hallberg et al., 1966). The following treatment groups were compared: (1) placebo (195 subjects) and ferrous sulfate (198 subjects; 222 mg elemental iron/day); (2) placebo (199 subjects), ferrous sulfate (120 subjects; 222 mg elemental iron/day), ferrous fumarate (118 subjects, 222 mg elemental iron/day), and ferrous gluconate (120 subjects; 222 mg elemental iron/day); and (3) placebo (200 subjects), ferrous sulfate (195 subjects; 180 mg elemental iron/day), ferrous glycine sulfate (200 subjects; 180 mg elemental iron/day), and ferrous gluconate (196 subjects; 180 mg elemental iron/day). Treatments were administered for two weeks. For all iron treatments, the frequency of adverse gastrointestinal effects was significantly increased compared to the matched placebo group ($p<0.05$). Adverse effects reported include constipation, diarrhea, heartburn, nausea and epigastric pain. No statistically significant differences in the frequency of adverse effects were observed between iron treatments for subjects receiving 222 mg elemental iron/day or between iron treatments for subjects receiving 180 mg elemental iron/day. In the seven iron treatment groups, the percentage of subjects reporting gastrointestinal effects ranged from 22.9% in the 222 mg ferrous sulfate group to 31.5% in the 222 mg ferrous gluconate group. In the three placebo treatment groups, the percentage of subjects reporting gastrointestinal effects ranged from 12.4 to 13.6%. Although statistical comparisons were not made between the 180 and 222 mg iron/day treatments, the frequency of adverse effects was similar for all iron treatment groups.

Gastrointestinal symptoms were reported in pregnant women treated daily with oral iron supplements containing 105 mg elemental iron and 500 mg ascorbic acid (55 women) or 105 mg

elemental iron, 500 mg ascorbic acid and 350 mg folic acid (54 women) during the third trimester of pregnancy (Blot et al., 1981). The form of iron was not reported. No placebo control group was included. Gastrointestinal adverse effects reported include nausea, diarrhea, constipation and epigastric pain. Approximately 16% of all patients reported minor gastrointestinal symptoms, 14% reported severe effects and 6% stopped treatment due to adverse effects. Adverse effects occurred with approximately the same frequency in the two treatment group, although data were not reported.

The tolerability of iron protein succinylate and ferrous sulfate were compared in a double-blind clinical trial in 1095 patients with iron deficiency (Liguori, 1993). Patients received daily treatment with a controlled-release formulation of ferrous sulfate containing 105 mg elemental iron (64 males and 485 females) or iron protein succinylate containing 120 mg elemental iron (55 males and 491 females) for 60 days. No placebo control group was included. In the ferrous sulfate group, 26.3% of patients reported adverse gastrointestinal effects (heartburn, epigastric pain, constipation and abdominal pain), compared to 11.5% of patients treated with iron protein succinylate ($p<0.05$).

The adverse effects of oral treatment with a conventional ferrous sulfate tablet were compared to a ferrous sulfate wax-matrix tablet in a single-blind, parallel group study in 543 subjects (Brock et al., 1985). No placebo control group was included. Subjects were administered a conventional ferrous sulfate table containing 50 mg elemental iron/day (272 subjects) or a sulfate wax-matrix tablet containing 50 mg elemental iron/day (271 subjects) for 56 days. Approximately 45% of subjects treated with conventional ferrous sulfate reported moderate-to-severe gastrointestinal effects, including abdominal discomfort, nausea, vomiting, constipation and diarrhea, compared to approximately 17% of subjects treated with the ferrous sulfate wax-matrix preparation, a statistically significant difference ($p<0.001$).

The tolerability of ferrous sulfate (50 mg elemental iron/day) and bis-glycino iron II (50 mg elemental iron/day) was compared in a double-blind, crossover trial in 42 women (Coplin et al., 1991). The treatment period for each iron supplement was two weeks. No placebo treatment period was included. The frequency of adverse gastrointestinal effects (abdominal pain, bloating, constipation, diarrhea and nausea) was similar for the two treatments, with 54% and 59% of subjects reporting gastrointestinal symptoms during treatment with bis-glycino iron II and ferrous sulfate, respectively. The difference between treatments was not statistically significant.

Effects of iron therapy on the upper gastrointestinal tract were evaluated in 14 healthy volunteers [13 women, 1 man; mean age 29 years (range: 24-48 years)] who were instructed to ingest 325 mg tablets of ferrous sulfate (119.5 mg elemental iron) three times/day before meals (358.5 mg elemental iron/day) for 2 weeks (Laine et al., 1988). Evaluation consisted of a gastrointestinal symptom survey, qualitative (Hemoccult) and quantitative (HemoQuant; mg mercury/g stool) testing for fecal blood loss, endoscopy of the upper gastrointestinal tract and histological examination of pinch biopsies of the gastric body, antrum and duodenum. Based on actual average ingestion of 2.5 tablets/day (2-week study) and 2.6 tablets/day (1-week study) and a reference human body weight of 70 kg (U.S. EPA, 1987), the estimated doses consumed by the subjects were 4.3 and 4.4 mg iron/kg-day, respectively, in addition to dietary iron. Compared to

baseline measurements in the two weeks prior to treatment, all subjects had significantly increased ($p<0.05$) dark brown-black stools and symptoms of nausea and vomiting during the treatment period, but not abdominal pain. Hemoglobin levels in stool did not change significantly after iron treatment. Endoscopic examination showed a significant ($p=0.003$) increase in abnormalities in the stomach, but not duodenum, after therapy. These changes consisted of erythema, small areas of subepithelial hemorrhage and solitary antral erosions in nine, six and two subjects, respectively, and were considered only minimally abnormal. No treatment-related histological changes were observed. Although it was speculated that the changes in the stomach could represent a mild form of iron poisoning, the investigators concluded that the treatment caused mild endoscopic abnormalities of uncertain clinical significance in the stomach. Evidence for iron overload (tissue biopsies or hematologic iron status indices) was not examined. Considering additional dietary exposure, an exposure level of about 4.3 mg/kg-day represents, at worst, a minimal LOAEL.

Adverse developmental effects in humans have not been associated with the ingestion of supplemental iron during pregnancy. As indicated above, NAS (2001) recommended that pregnant women supplement their diets with 27 mg iron/day (0.35 mg/kg-day). McElhatton et al. (1991) reported on 49 women who took an overdose of a simple iron preparation (53%) or iron with folate preparation (47%). In 48 of the women, the amount of iron ingested was known; 28 took > 1.2 g and the remainder took $\leq$ 1.2 g. There were 25 women who received chelation treatment with desferrioxamine (DFO) and 12 who received an emetic. Maternal toxicity, consisting of nausea, vomiting, hematoemesis, abdominal pain and diarrhea, was observed in 35 of the women. Two spontaneous abortions occurred and there were three premature deliveries. One of the spontaneous abortions and the premature deliveries were not related to the iron overdose. It is not known if the other spontaneous abortion occurring at 22 weeks (3 weeks after the overdose) was caused by the iron overdose. No conclusions on the developmental toxicity of iron can be made.

Chronic Exposure

While chronic iron toxicity occurs in people with genetic metabolic disorders resulting in excessive iron absorption or abnormal hemoglobin synthesis, or who receive frequent blood transfusions (Jacobs, 1977; Bothwell et al., 1979), there is a long-standing controversy as to whether a chronic overload due to oral intake is possible in individuals with a normal ability to control iron absorption (Hillman and Finch, 1985). Nevertheless, "the cumulative experience in human subjects suffering from iron overload of various etiologies strongly suggests that iron is noxious to tissues [when]...present in parenchymal cells...for a sufficiently long period of time" (Bothwell et al., 1979).

Looker et al. (1988) made comparisons of dietary iron intake and biochemical indices of iron status based on values taken from the second National Health and Nutrition Examination Survey (NHANES II) data base[1]. NHANES II was a probability sample of the noninstitutionalized U.S. population aged 6 months to 74 years, conducted between 1976 and

[1] The latest version of this data base, NHANES III (1984-1988) evaluated 30,000 subjects aged 2 months and above (NAS, 2001). Despite minor differences in the data sets, the conclusions drawn by Looker et al. (1988) based on NHANES II appear to be valid for the NHANES III data.

1980 by the National Center for Health Statistics. These data suggest that normal intake of iron by men 16-74 years old exceeds the DRI, and that iron intake is somewhat lower than the DRI for women younger than 51 years. Concomitant with the study of dietary intake, the NHANES II measured the iron status of these populations. The percent serum transferrin saturation, a measure of the residual capacity of the iron transport system to process potential variations in iron from dietary intake or catabolized body stores, ranged from 24% saturation for pre- and post-menopausal women not using iron supplements to 29% saturation for adult male supplement users. These values are within the normal range (20-40%). The Looker et al. (1988) evaluation of the NHANES II iron status data concerned iron deficiencies, only, and did not address iron overload directly. However, iron overload conditions would likely be evidenced by increased saturation of serum transferrin and increased serum ferritin concentrations, which were also within the normal range. Therefore, the corresponding dietary intakes are presumed to represent chronic NOAELs. Looker et al. (1988) estimated daily iron intakes ranging from 10.0 for elderly women to 18.7 mg/day for young adult men in the study population. These daily intakes correspond to a range of about 0.15 to 0.27 mg/kg-day, depending on assumptions of average body weight. Taking the highest intake level of 18.7 mg/day and a body weight of 70 kg, a NOAEL of 0.27 is established for chronic iron toxicity.

Hemosiderosis (or siderosis) and iron overload are increases in tissue iron or a general increase in iron stores without associated tissue damage (Bothwell et al., 1979; Jacobs, 1977). Hemochromatosis describes massive iron overload (15 g of body iron stores or greater) together with cirrhosis and/or other tissue damage attributable to iron. Although focal deposits of iron may occur in any part of the body where red cells are extravasated, the clinical syndrome of hemochromatosis typically involves damage to the hepatic parenchyma (particularly fibrosis), heart (cardiac dysfunction including failure) and endocrine glands (particularly hypogonadism). Pancreatic iron deposition is common and massive deposits may be associated with fibrosis and diabetes. A number of studies involving chronic oral administration of iron to animals have been designed in an attempt to identify an animal model for hemochromatosis. Most of these studies have been negative (Bothwell et al., 1979; NRC, 1979). Animal studies involving parenteral administration of iron have been generally negative as well, even though parenteral routes bypass the mechanisms that regulate absorption of iron from the gastrointestinal tract.

Chronic iron toxicity has been observed in people with idiopathic hemochromatosis (a genetic metabolic disorder resulting in excessive iron absorption), abnormalities of hemoglobin synthesis (e.g., thalassemia) or various anemic states (e.g., sideroblastic anemia), frequent blood transfusions or a combination of these conditions (Jacobs, 1977; Bothwell et al., 1979). Chronic hemochromatosis has also occurred among the South African Bantu population from an excessive intake of absorbable iron in an alcoholic beverage.

Habitual excessive intake of iron by the Bantus is attributed to consumption of home-brewed Kaffir beer, which was contaminated by iron vessels during brewing (Bothwell and Bradlow, 1960; Bothwell et al., 1964). The beer's high acidity (pH 3-3.5) enhanced iron leaching from the vessels. The iron in the beer is readily assimilable (i.e., ionizable) due to the acidity and presence of iron-complexing ligands such as fructose, and is absorbed to approximately the same degree as ferric chloride. The alcohol content of the beer is also believed to contribute to the bioavailability of the iron (Jacobs, 1977; Finch and Monsen, 1972). Based

primarily on drinking habits and analyses of beer samples, the estimated average dietary iron intake of the Bantu men ranged from 50-100 mg/day from beer alone (Bothwell et al., 1964). Using a reference body weight of 70 kg (U.S. EPA, 1987), this range corresponds to 0.7-1.4 mg/kg-day. Histological examinations of the liver of 147 Bantus (129 male, 18 female) ranging in age from 11-70 years (most were between 20 and 50 years old) that died from acute traumatic causes were performed (Bothwell and Bradlow, 1960). Varying degrees of hepatic siderosis were observed in 89% of the cases; the degree tended to increase with age 40-50 years or less. The siderosis was mild in 59% and severe in 19% of the cases, respectively. There was a close correlation between hepatic iron concentration and portal fibrosis and cirrhosis. Although the overall prevalence was low (15.6% fibrosis and 1.4% cirrhosis), all 11 subjects with the highest iron concentrations (>2.0% dry weight of liver) showed either fibrosis or cirrhosis. Histological examination of the spleen (50 subjects) also showed siderosis and unspecified histological changes. Malnutrition and alcoholism could have played a role in the etiology of the hepatic and splenic siderosis in the Bantus. A NOAEL in the range of 0.7 - 1.4 mg/kg-day is indicated but may be low given the likely higher bioavailability of iron in the beer than for normal dietary exposure. Given the generally poor nutritional health status of this population, the relevance of this study for application to the U.S. population is questionable.

Ethiopia reportedly has the highest per capita iron intake in the world, with an average daily intake of 471 mg iron/day (range 98-1418 mg/day; 1.4-20.3 mg iron/kg-day assuming 70 kg body weight) (Roe, 1966; Hofvander, 1968). Increased stored iron in the liver and adverse health effects have not been observed due to low bioavailability of the iron in Ethiopian food.

A few studies have suggested that high iron intake may be a risk factor for myocardial infarction (Salonen et al., 1992; Lauffer, 1991; Sullivan, 1992). Five other large studies found no association between serum ferritin levels and coronary heart disease (NAS, 2001). Various other measures of iron status (serum transferrin saturation, serum iron concentration and total iron-binding capacity) have been examined for a possible link to cardiovascular disease in prospective cohort studies, but results overall have been characterized as contradictory (Meyers, 1996; NAS, 2001). The NAS (2001) concluded that the available evidence "does not provide convincing support for a causal relationship" between the level of dietary iron intake and the risk for coronary heart disease, although iron cannot be definitively excluded as a risk factor.

Animal Studies

Repeated-dose oral studies in experimental animals found no significant effect of treatment with inorganic iron compounds. No treatment-related adverse changes in clinical signs, body or organ weights, food consumption or histopathology were observed in male Sprague-Dawley rats that had daily dietary intakes of 35, 70 or 140 mg of iron (as $FeSO_4$ or FeEDTA) per kg for up to 61 days (Appel et al., 2001). In male and female F344 rats that were exposed to drinking water containing 0.25 or 0.5% ferric chloride ($FeCl_3 \cdot 6H_2O$) for 104 weeks, there were no dose-related effects other than reduced water intake (possibly affected by palatability) and body weight gain (Sato et al., 1992). In the latter study, the iron intakes were 58 or 110 mg/kg-day in males and 65 or 116 mg/kg-day in females.

No treatment-related teratogenic or embryotoxic effects were observed in rats given 2.7 mg iron/kg-day as ferric chloride on gestational days 6-15 (Nolen et al., 1972), or in rats and mice given 24-76 mg iron/kg-day as ferrous sulfate for 6 days during gestation (days unspecified) (Tadokoro et al., 1979). Some embryonic mortality (numbers and species not reported) occurred in the latter study at 240 mg iron/kg-day.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR IRON

Iron is an essential element, as such, the RfD must be protective against both toxicity and deficiency. Using the values for dietary intake and iron status indices taken from the second National Health and Nutrition Examination Survey (NHANES II) data base, it is possible to establish a NOAEL for chronic toxicity. Looker et al. (1988) made comparisons of dietary iron intake and biochemical indices of iron status using data from NHANES II. The average intakes of iron ranged from 0.15 to 0.27 mg/kg-day. The serum ferritin levels and percent serum transferrin saturation were within the normal range. Thus, intake levels of 0.15-0.27 mg/kg-day are sufficient to protect against iron deficiency. However, the NHANES II data do not provide information to identify daily dietary iron intakes associated with toxicity. Therefore, daily dietary iron intakes were not considered as the basis for the p-RfD.

Most of the quantitative chronic oral toxicity data for iron have been obtained from studies of the Bantu population of South Africa. These data indicate that intakes in the range of 0.7-1.4 mg iron/kg-day in home-brewed beer are associated with hemosiderosis and liver cirrhosis (Bothwell and Bradlow, 1960; Bothwell et al., 1964). However, confounding factors such as malnutrition and unusually high iron bioavailability due to the high acidity and ethanol in the beer preclude use of these data for risk assessment. Much higher dietary intakes (average 6.7 mg/kg-day) of less soluble forms of iron are tolerated in non-western diets as indicated by studies of populations in Ethiopia. Thus, although toxicity associated with iron overload due to chronic oral intake can be demonstrated qualitatively or even semiquantitatively, assignment of a precise LOAEL for normal individuals consuming western diets is compromised by studies containing confounding factors.

Gastrointestinal toxicity, which is commonly associated with the therapeutic use of iron supplements, was identified as the critical effect for the basis of the provisional subchronic and chronic RfDs. The most frequently reported symptoms include epigastric pain, nausea, vomiting, constipation and diarrhea. Several prospective clinical trials in healthy subjects and iron-deficient patients identify a LOAEL for gastrointestinal toxicity of 50 to 180 mg elemental iron/day; NOAELs were not established (Blot et al., 1981; Brock et al., 1985; Coplin et al., 1991; Frykman et al., 1994; Hallberg et al., 1966; Liguori, 1993). The treatment durations in these studies range from 2 weeks to approximately 3 months. Although no chronic exposure studies reporting gastrointestinal toxicity were identified, clinical experience with iron supplements indicates that gastrointestinal effects are associated with oral iron therapy, regardless of the duration of treatment and that symptom intensity does not change over the course of treatment (Hillman, 2001; Santi and Masters, 2001). This observation suggests that the response is related to the concentration of iron in the intestinal tract and not to the time-integrated dose. Therefore,

gastrointestinal toxicity is considered as the critical effect for both the subchronic and chronic p-RfDs.

The lowest LOAEL of 50 mg elemental iron/day for gastrointestinal toxicity associated with iron supplements was reported in two studies that did not use a placebo-controlled design (Brock et al., 1985; Coplin et al., 1991); therefore, data were not considered suitable for derivation of the p-RfD. The placebo-controlled, cross-over design study by Frykman et al. (1994) reporting a LOAEL of 60 mg/day in Swedish men and women was identified as the critical study. Results of this study show that daily treatment with ferrous fumarate (60 mg elemental iron/day) for one month produced a statistically significant increase in gastrointestinal effects compared to placebo. To determine the LOAEL for total daily iron intake, the LOAEL for daily supplementation with ferrous fumarate of 60 mg elemental iron/day was added to the estimated mean dietary intake for six European countries of 11 mg elemental iron/day (NAS, 2001) for a total daily iron intake of 71 mg elemental iron/day. Based on a reference body weight of 70 kg (U.S. EPA, 1987), the LOAEL for gastrointestinal effects for total daily iron intake is 1 mg elemental iron/kg-day. This LOAEL is considered to be a minimal LOAEL because gastrointestinal effects were characterized by most study participants as minor in severity.

The provisional subchronic and chronic RfD for iron was derived from the LOAEL of 1 mg/kg-day for total daily iron intake for adverse gastrointestinal effects as follows:

$$\begin{aligned} \textbf{p-RfD (subchronic and chronic)} &= \textbf{LOAEL} \div \textbf{UF} \\ &= \textbf{1 mg/kg-day} \div \textbf{1.5} \\ &= \textbf{0.7 mg/kg-day} \end{aligned}$$

Dividing the LOAEL of 1 mg/kg-day by an uncertainty factor of 1.5 yields a subchronic and chronic p-RfD of 0.7 mg/mg-day. The uncertainty factor of 1.5 includes the individual uncertainty factors of 1.5 for use of a minimal LOAEL, 1 for sensitive individuals, 1 for less than lifetime exposure, and 1 for an adequate data base. An uncertainty factor of 1.5 was applied to account for extrapolation from a minimal LOAEL to a NOAEL for a non-serious effect. A higher uncertainty factor for use of a minimal LOAEL was not used since the observed gastrointestinal effects are not considered serious and are reversible when exposure is discontinued. Furthermore, gastrointestinal symptoms are not associated with dietary intake of similar levels of iron (NAS, 2001). Because individuals sensitive to gastrointestinal symptoms are considered to be included in the studies investigating effects of therapeutic iron; an uncertainty factor of 1 for sensitive individuals results. An uncertainty factor of 1 was used to account for less than lifetime exposure. Although exposure duration in the Frykman et al. (1994) study was only one month, there is no evidence to suggest that symptoms increase with longer exposure periods. An uncertainty factor of 1 was used to reflect an adequate database in humans, due to the extensive use of therapeutic iron.

Except for individuals with disorders of iron metabolism, little information is available on the long-term systemic toxicity of orally ingested iron. This assessment, therefore, focuses more on what is known to be a safe oral intake of iron for the general human population (i.e., apparently healthy normal individuals). The provisional reference dose is estimated to be an

intake for the general population that is adequately protective from adverse health effects. Further, it is also important to note that individual requirements for, as well as adverse reactions to, iron may be highly variable. Some individuals may, in fact, consume a diet that contributes more than the provisional reference dose, without any cause for concern. In addition, specific population subgroups may have higher nutritional requirements than the provisional RfD would provide. The p-RfD may not be protective of individuals with inherited disorders of iron metabolism or other conditions which affect iron homeostasis.

This assessment is essentially the same as that proposed by Stifelman et al. (2005).

REFERENCES

Appel, M.J., C.F. Kuper and R.A. Woutersen. 2001. Disposition, accumulation and toxicity of iron fed as iron (II) sulfate or as sodium iron EDTA in rats. Food Chem. Toxicol. 39:261-269.

Arena, J.M. 1970. Poisoning, 2nd ed. Thomas, Springfield, IL. p. 369. (Cited in Crotty, 1971)

ATSDR (Agency for Toxic Substances and Disease Registry). 2005. Toxicological Profile Query. Available at http://www.atsdr.cdc.gov/toxprofiles/.

Banner Jr., W. and T.G. Tong. 1986. Iron poisoning. Pediatr. Toxicol. 33(2):393-409.

Blot, I., J.P. Kaltwasser, E.Werner and G. Tchernia. 1981. Influence of routine administration of folic acid and iron during pregnancy. Gynecol. Obstet. Invest. 12:294-304.

Bothwell, T.H. and B.A. Bradlow. 1960. Siderosis in the Bantu: A combined histopathological and chemical study. Arch. Pathol. 70:279-292.

Bothwell, T.H., H. Seftel, P. Jacobs et al. 1964. Iron overload in Bantu subjects: Studies on the availability of iron in Bantu beer. Am. J. Clin. Nutr. 14:47-51.

Bothwell, T.H., R.W. Charlton, J.D. Cook and C.A. Finch. 1979. Iron Metabolism in Man. Blackwell Scientific Publications, Oxford, UK. p. 7-43, 105-120, 256-283.

Brock, C., H. Curry and C. Hanna. 1985. Adverse effects of iron supplementation: a comparative trial of a wax-matrix preparation and conventional ferrous sulfate tablets. Clin. Therap. 7(5):568-577.

CDC (Centers for Disease Control and Prevention). 1998. Recommendations to prevent and control iron deficiency in the United States. MMWR Recomm Rep. 47:1-29 (on-line)

CDC (Centers for Disease Control and Prevention). 2005. Anemia and Iron Status. http://www.cdc.gov/nccdphp/dnpa/anemiron.htm

Cook, J.D. 1991. Telephone conversation with J.A. Freedman, Syracuse Research Corporation, Syracuse, NY. Discussion of normal ranges of indices of iron status. September 5, 1991.

Coplin M., S. Schuette, G. Leichtmann and B. Lashner. 1991. Tolerability of iron: A comparison of bis-glycion iron II and ferrous sulfate. Clin. Therap. 13(5):606-612.

Elinder, C. 1986. Iron. In: Handbook on the Toxicology of Metals, 2nd ed., vol. II. L. Friberg, G.F. Nordberg, V.B. Vouk and E. Kessler, Eds. Elsevier Science Publishers, New York. p. 276-297.

Engle, J.P., K.S. Polin and I.L. Stile. 1987. Acute iron intoxication: Treatment controversies. Drug Intell. Clin. Pharm. 21:153-159.

Finch, C.A. and E.R. Monsen. 1972. Iron nutrition and the fortification of food with iron. J. Am. Med. Assoc. 219:1462-1465.

Frykman, E., M. Bystrom, U. Jansson, A. Edberg and T. Hansen. 1994. Side effects of iron supplements in blood donors: Superior tolerance of heme iron. J Lab Clin Med. 123(4):561-4.

Grimsley, L.F. 2001. Iron and cobalt. In: Patty's Toxicology, 5th edition, vol. 3, E. Bingham, B. Cohrssen and C.H. Powell, Eds. John Wiley and Sons, New York. p. 169-193.

Hallberg, L., L. Ryttinger and L. Solvell. 1966. Side-effects of oral iron therapy. Acta Med. Scand. Suppl. 459:3-10.

Hillman, R.S. and C.A. Finch. 1985. Drugs effective in iron-deficiency and other hypochromic anemias. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th ed. MacMillan Publishing Company, New York. p. 1308-1322.

Hillman, R.S. 2001. Hematopoietic Agents: Growth Factor, Minerals and Vitamins. In: Goodman and Gilman's The Pharmacological Basis of Therpeutics. J.G. Hardman, L.E. Limbird and A.G. Gilman, Eds. McGraw-Hill Companies, Inc., Medical Publishing Division, New York. p. 1487-1517.

Hofvander, Y. 1968. Hematological investigations in Ethiopia with special reference to a high iron intake. Acta Med. Scand. Suppl. 494:7-74.

IARC (International Agency for Research on Cancer). 1972. Haematite and iron oxide. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 1. Lyon, France. pp. 29-39.

IARC (International Agency for Research on Cancer). 1987. Haematite and ferric oxide. Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs* Volumes 1 to 42. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Suppl. 7. Lyon, France. pp. 216-219.

Jacobs, A. 1977. Iron overload – clinical and pathologic aspects. Semin. Hematol. 14:89-113.

Klein-Schwartz, W., G.M. Oderda, R.L. Gorman et al. 1990. Assessment of management guidelines: Acute iron ingestion. Clin. Pediatr. 29(6):316-321.

Krenzelok, E.P. and J.V. Hoff. 1979. Accidental childhood iron poisoning: A problem of marketing and labeling. Pediatrics. 63(4):591-596.

Laine, L.A., E. Bentley and P. Chandrasoma. 1988. Effect of oral iron therapy on the upper gastrointestinal tract. A prospective evaluation. Digest. Dis. Sci. 33(2):172-177.

Lauffer, R.B. 1991. Iron stores and the international variation in mortality from coronary artery disease. Med. Hypoth. 35(2):96-102.

Liguori, L. 1993. Iron protein succinylate in the treatment of iron deficiency: controlled, double-blind, multicenter clinical trial on over 1,000 patients. Int. J. Clin. Pharmacol. Ther. Toxicol. 31(3):105-123.

Looker, A., C.T. Sempos, C. Johnson and E.A. Yetley. 1988. Vitamin-mineral supplement use: Association with dietary intake and iron status of adults. J. Am. Diet. Assoc. 88:808-814.

Mann, K.V., M.A. Picciotti, T.A. Spevack and D.R. Durbin. 1989. Management of acute iron overdose. Clin. Pharm. 8(6):428-440.

McElhatton, P.R., J.C. Roberts and F.M. Sullivan. 1991. The consequences of iron overdose and its treatment with desferrioxamine in pregnancy. Hum. Exp. Toxicol. 10:251-259.

Meyers, D.G. 1996. The iron hypothesis–Does iron cause atherosclerosis? Clin. Cardiol. 19:925-929.

NAS (National Academy of Sciences). 1989. Recommended Dietary Allowances, 10th ed. National Academy of Sciences, National Research Council, Food and Nutrition Board. National Academy Press, Washington, DC. p. 195-205.

NAS (National Academy of Sciences). 2001. Iron. In: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Institute of Medicine, Food and Nutrition Board. National Academy Press, Washington, DC. p. 233-310. Available at http://books.nap.edu/books/0309072794/html/.

Nolen, G.A., R.L. Bohne and E.V. Buehler. 1972. Effects of trisodium nitrilotriacetate, trisodium citrate, and a trisodium nitrilotriacetate ferric chloride mixture on cadmium and methyl mercury toxicity and teratogenesis in rats. Toxicol. Appl. Pharmacol. 23:238-250.

NRC (National Research Council). 1979. Iron Metabolism in Humans and Other Mammals. A Report of the Subcommittee on Iron, Committee on Medical and Biologic Effects of Environmental Pollutants, Division of Medical Sciences, Assembly of Life Sciences. University Park Press, Baltimore, MD. p. 79-105, 33-165.

NTP (National Toxicology Program). 2001. NTP Chemical Repository Report for Ferric Chloride. Available at http://ntp-server.niehs.nih.gov/htdocs/CHEM_H&S/NTP_Chem7/Radian7705-08-0.html.

NTP (National Toxicology Program). 2005. Management Status Report. Available at http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/Res_Stat/iH_Res_Stat_Frames.html.

Roe, D.A. 1966. Nutrient toxicity with excessive intake. II. Mineral overload. N.Y.S. J. Med. 66:1233-1237.

Salonen, J.T., K. Nyyssonen, H. Korpela, J. Tuomilehto, R. Seppanen and R. Salonen. 1992. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. Circulation. 86(3):803-811.

Santi, D.V. and S.B. Masters. 2001. Agents Used in Anemias; Hematopoietic Growth Factors. In: Basic & Clinical Pharmacology. B.G. Katzung, Ed. Lange Medical Books/McGraw-Hill Companies, Inc., Medical Publishing Division, New York. p. 549-563.

Sato, M., F. Furukawa, K. Toyoda et al. 1992. Lack of carcinogenicity of ferric chloride in F344 rats. Food Chem. Toxicol. 30:837-842.

Stifelman, M., L. Ingerman, B. Thayer and G. Diamond. 2005. Risk Assessment for Iron: Use of the Institute of Medicine's Tolerable Upper Intake Level as a Surrogate Toxicity Value for Iron. Presented at the Society of Toxicology Annual Meeting, March 6, 2005, New Orleans, LA.

Sullivan, J.L. 1992. Stored iron and ischemic heart disease. Empirical support for a new paradigm [editorial; comment]. Circulation. 36(3):1036-1037.

Tadokoro, T., T. Miyaji and M. Okumura. 1979. Teratogenicity studies of slow-iron in mice and rats. Oyo Yakuri. 17:483. (Taken from Chem. Abstr. 91:134052f)

U.S. EPA. 1984. Health Effects Assessment for Iron (and Compounds). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. EPA/540/1-86/054.

U.S. EPA. 1987. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC. EPA/600/6-87/008. NTIS PB88-179874/AS.

U.S. EPA. 1991. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. Annual Update. FY-1997. Office of Research and Development, Office of Emergency and Remedial Response, Washington, D.C. July 1997. EPA/540/R-97/036. NTIS PB97-921199.

U.S. EPA. 2005. Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. Available at http://www.epa.gov/ost/drinking/standards/dwstandards.pdf.

U.S. EPA. 2006. Integrated Risk Information System (IRIS). Online. Office of Research and Development. National Center for Environmental Assessment, Washington, D.C. www.epa.gov/iris.

U.S. FDA. 1997. Iron-Containing Supplements and Drugs: Label Warning Statements and Unit-Dose Packaging Requirements; Final Rule. Fed. Reg. 62(10):2217:2250.

U.S. FDA. 2003. Iron-Containing Supplements and Drugs; Label Warning Statements and Unit-Dose Packaging Requirements; Removal of Regulations for Unit-Dose Packaging Requirements for Dietary Supplements and Drugs. Fed. Reg. 68(201):59714-59715.

WHO (World Health Organization). 2005. Online catalogs for the Environmental Health Criteria series. Available at http://www.who.int/dsa/cat97/zehc.htm and http://www.who.int/dsa/justpub/add.htm.

6-16-05

Provisional Peer Reviewed Toxicity Values for

Iron and Compounds
(CASRN 7439-89-6)

Derivation of a Chronic Inhalation RfC

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	**provisional inhalation unit risk**
p-OSF	**provisional oral slope factor**
p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

6-16-05

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR IRON (CASRN 7439-89-6) AND COMPOUNDS
Derivation of an Inhalation RfC

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

An RfC for iron is not listed on IRIS (U.S. EPA, 2001) and was not considered by the RfD/RfC Work Group (U.S. EPA, 1995). The HEAST (U.S. EPA, 1997) reported that data regarding iron were inadequate for quantitative risk assessment. The CARA list (1991, 1994a) includes a Health Effects Assessment for Iron and Compounds (U.S. EPA, 1984) that reported negative epidemiological studies (no association between excess mortality or respiratory diseases and occupational exposure to iron oxide dusts) and no available subchronic or chronic inhalation studies in animals. In March, 2004, a literature search was also conducted using TOXLINE, MEDLINE, Chemical Abstracts and Biological Abstracts data bases.

Occupational exposure limits have been established for soluble iron salts and iron oxide, as well as for organic iron compounds not covered in this issue paper. The ACGIH (1991a,

2001) has adopted a TLV-TWA, NIOSH (2001a) has established a REL-TWA, and OSHA (2001a, 2001b) has adopted a construction industry PEL-TWA of 1 mg/m^3, as Fe, to reduce the likelihood of irritation to eyes, skin, and respiratory tract from exposure to aerosols or mists of soluble iron salts (ferrous and ferric sulfates and chlorides, and ferric nitrate). The ACGIH (1991b, 2001) has adopted a TLV-TWA and NIOSH (2001b) has established a REL-TWA of 5 mg/m^3, as Fe, for dust and fume of ferric oxide (Fe_2O_3) to protect against siderosis, a benign pneumoconiosis. OSHA (2001c) has adopted a PEL-TWA of 10 mg/m^3 for ferric oxide fume, to protect against accumulation of iron dust in the lungs.

Iron has not been the subject of a toxicological profile by ATSDR (2001) or the WHO (2001). Monographs by IARC (1972, 1984, 1987), a toxicity review on iron (Grimsley, 2001), and the NTP (2001a, 2001b) management status report and chemical repository summary were consulted for information relevant to inhalation toxicity of iron and inorganic iron compounds. The following computer searches, performed in April, 1993, were screened to identify additional pertinent studies not discussed in review documents: TOXLINE (1983-April, 1993), CANCERLIT (1990 - April, 1993), MEDLINE (1991 - April, 1993), TSCATS, RTECS, and HSDB. Update literature searches were conducted in September, 2001 in TOXLINE (1992-September, 2001), CANCERLIT (1992- September, 2001), MEDLINE (1992-September, 2001), TSCATS, RTECS, DART/ETICBACK, EMIC/EMICBACK, HSDB, GENETOX, and CCRIS.

REVIEW OF PERTINENT LITERATURE

Human Studies

A number of studies have examined the relationship between respiratory disease and inhalation exposure to iron compounds for workers employed in hematite mining or other iron-related occupations, such as welding or steel-making (U.S. EPA, 1984; IARC, 1972, 1984; Grimsley, 2001). However, since these studies involved concurrent exposure to silica and other metals, they are not suitable for the health risk assessment of iron or iron compounds. The literature search did not discover any studies that examined subchronic or chronic inhalation exposures of humans to quantified levels of iron or iron compounds alone.

In a case-control study of cancer incidence, a Swedish male worker population (1958-1971) was reported to have had a high exposure to iron oxides from the production of sulfuric acid from pyrite (FeS_2) (Axelson and Sjöberg, 1979). The workers were exposed to iron oxide (Fe_2O_3) along with 1-2% copper, 0.01-0.1% arsenic, nickel and cobalt as impurities. Exposure in the workroom was estimated as approximately 50-100 mg/m^3, and the particle size as 25% below 10 μm and 5-10% below 5 μm. However, there were no measurements of exposure levels or particle size, and exposure durations were not reported. No cases of siderosis were known from the plant.

Animal Studies

Inhalation studies for iron compounds in animals include a chronic study of hamsters exposed to ferric oxide (Fe_2O_3) dust (Nettesheim et al., 1975) and a 2-month study in rabbits exposed to aerosols of ferric chloride (Johansson et al., 1992).

In a cancer study, groups of male Syrian hamsters (132 per group) were exposed to filtered air or Fe_2O_3 (analytic grade) dust at a concentration of 40 mg/m^3, 6 hours/day, 5 days/week for life (Nettesheim et al., 1975). The particle size had a geometric mean diameter of 0.11 μm. In addition, two satellite groups (15 hamsters per treatment) were sacrificed, three animals at a time, at 2, 4, 8, 12, and 104 weeks, so that the accumulation of iron in the lung from inhaled Fe_2O_3 could be compared to background iron concentrations in heme. The animals were examined daily, before and after each exposure, for clinical signs, and body weights were recorded monthly. All animals except those cannibalized (<2%) were necropsied. Histological analyses were performed on the major organs, including heart, trachea, lungs, and nasal cavities. Examination of the satellite groups demonstrated the gradual increase in iron accumulation in the lung, reaching a total of 10 mg per lung at 104 weeks. Histological examination revealed iron deposits in the lungs and tracheal and bronchial lymph nodes of all exposed animals. Diffuse and focal alveolar fibrosis was also frequently observed in the lungs of treated animals. Results for the histological endpoints were not reported quantitatively. In this study, 40 mg/m^3 is a LOAEL for respiratory effects (alveolar fibrosis) in hamsters exposed to Fe_2O_3 dust.

Groups of 8 male rabbits (strain not reported) were exposed to aerosols of 0, 1.4, or 3.1 mg/m^3 of iron as $FeCl_3$ 6 hours/day, 5 days/week for 2 months (Johansson et al., 1992). At termination, the upper left lung lobe was examined by light microscopy, pieces of the lower left lung were analyzed by electron microscopy or used for phospholipid analysis, and the right lung was lavaged to obtain macrophages for morphological and functional analyses. The mass median aerodynamic diameter of the aerosols was ~1 μm as measured with an impactor. Treatment had no effect on survival. Lungs were spotted with black in 7/8 high-iron rabbits, in 2/8 low-iron rabbits, and in 0/8 controls. The absolute weight of the left lower lobe of the lung was significantly elevated compared to controls in the high-iron group. Exposure-related histopathology was observed in the lungs. In the high-exposure group, the lungs contained naked granulomas [large nodules (≥ 1 mm) of densely packed granular macrophages], accumulations of granular macrophages in terminal bronchioles, and foci of interstitial lymphocytic inflammatory reaction. Small granulomas were observed in one low-iron and one control rabbit. Accumulations of normal and granular macrophages were observed in the alveoli of exposed rabbits. In the control group, normal lung tissue contained some small accumulations of macrophages with occasional small inflammatory reaction. The high exposure group had a significantly higher density of alveolar type II cells than the controls. Ultrastructural analysis of macrophages showed a significantly higher number of abnormal cells, cells with enlarged lysosomes, and black inclusions in cells in both exposed groups; the high-iron group had higher

percentages of cells with laminar inclusions or with smooth cell surfaces. In functional tests, macrophages from the high-exposure group showed significantly elevated phagocytic activity, but no significant increase in oxidative metabolic activity (superoxide generation). Total phospholipids were elevated in the high-exposure group, but, as indicated by the lack of increase in phosphatidyl cholines or the percentage of 1,2-dipalmitoylphosphatidylcholine, the amount of surfactant was unchanged. In this study, the low concentration of 1.4 mg/m^3 is a NOAEL and the high concentration of 3.1 mg/m^3 is a LOAEL for adverse lung effects (nodular granulomas ≥ 1 mm in diameter, abnormal macrophages) in rabbits exposed to ferric chloride aerosols. Because of its focus on alveolar macrophage effects, this study provided no information regarding clinical signs of toxicity, body weight changes, clinical biochemistry, nasopharyngeal effects or histology of any other tissue besides the lung.

Other Studies

In a cancer study, groups of Syrian golden hamsters (24 per sex per group) received intratracheal instillations of 0 or "a maximum dose"[1] of 3 mg of Fe_2O_3 dust in 0.2 ml of saline once a week for 15 weeks, and then were observed up to week 120 (Stenbäck et al., 1976). Analysis by the sedimentation method demonstrated that 98% of the particles were less than 10 µm in diameter. Animals were weighed weekly and autopsied. Organs with gross lesions and the larynx, trachea, bronchi, and lungs were examined histologically. Treatment with ferric oxide had no effect on survival and no effect on body weight except during the final weeks of survival (data not shown). Deposited iron oxide was grossly visible as dark patches on the lung surface. Histologically, dust accumulations surrounded by cellular infiltrates were observed in the peribronchial region. Interstitial fibrosis was observed occasionally, but distinct inflammatory changes were rare. Results for the nonneoplastic endpoints were not reported quantitatively.

FEASIBILITY OF DERIVING A PROVISIONAL RfC FOR IRON

No adequate human or animal inhalation data are available for exposure to iron or inorganic iron compounds. The epidemiological study of Axelson and Sjöberg (1979) did not provide quantitative measures of exposure and did not characterize noncancer endpoints. Although Nettesheim et al. (1975) reported diffuse and focal alveolar fibrosis in the lungs of hamsters chronically exposed to iron oxide by inhalation at a concentration of 40 mg/m^3, the lack of incidence data prevents an evaluation of the significance of these findings. The subchronic study of Johansson et al. (1992), in which rabbits were exposed to aerosols of ferric chloride for

[1]The authors provided no further information regarding dosage. It is not clear whether animals were given amounts lower than 3 mg on some occasions.

2 months, demonstrated a NOAEL of 1.4 mg/m^3 and a LOAEL of 3.1 mg/m^3 for respiratory effects (granuloma nodules greater than 1 mm diameter in the lungs). However, this study does not meet the minimum standards for an inhalation bioassay as stipulated by the U.S. EPA (1994b) guidelines for derivation of an inhalation reference concentration. Inadequacies of the study include relatively small group sizes, relatively short study duration, and the failure to examine a sufficient array of endpoints. Thus this study is inadequate for the purposes of deriving a p-RfC for iron. Consequently, the available data are insufficient for derivation of a p-RfC.

REFERENCES

ACGIH (American Conference of Government Industrial Hygienists). 1991a. Iron Salts (Soluble). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. ACGIH. Cincinnati, OH. p. 808-809.

ACGIH (American Conference of Government Industrial Hygienists). 1991b. Iron Oxide, CAS: 1309-37-1. Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. ACGIH. Cincinnati, OH. p. 803-805.

ACGIH (American Conference of Government Industrial Hygienists). 2001. Threshold limit values (TLV) for chemical substances and physical agents and biological exposure indices. ACGIH, Cincinnati, OH. p. 36.

ATSDR (Agency for Toxic Substances and Disease Registry). 2001. Internet HazDat - Toxicological Profile Query. Examined September, 2001. Online at http://www.atsdr.cdc.gov/gsql/toxprof.script.

Axelson, O. and A. Sjöberg. 1979. Cancer incidence and exposure to iron oxide dust. J. Occup. Med. 21: 419-422.

Grimsley, L.F. 2001. Iron and cobalt. In: Patty's Toxicology, 5th Edition, vol. 3, E. Bingham, B. Cohrssen and C.H. Powell, Eds. John Wiley and Sons, New York. p. 169-193.

IARC (International Agency for Research on Cancer). 1972. Haematite and iron oxide. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 1, pp. 29-39. Lyon, France.

IARC (International Agency for Research on Cancer). 1984. Iron and steel founding. Polynuclear Aromatic Compounds, Part 3, Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 34, pp. 133-190. Lyon, France.

IARC (International Agency for Research on Cancer). 1987. Haematite and ferric oxide. Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs* Volumes 1 to 42. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Suppl. 7, pp. 216-219. Lyon, France.

Johansson, A., T. Curstedt, O. Rasool et al. 1992. Macrophage reaction in rabbit lung following inhalation of iron chloride. Environ. Res. 58: 66-79.

Nettesheim, P., D.A. Creasia and T.J. Mitchell. 1975. Carcinogenic and cocarcinogenic effects of inhaled synthetic smog and ferric oxide particles. J. Natl. Cancer Inst. 55: 159-169.

NIOSH (National Institute for Occupational Safety and Health). 2001a. Iron salts (soluble, as Fe). NIOSH Pocket Guide to Chemical Hazards. September, 2001. Online at http://www.cdc.gov/niosh/npg/npgd0346.html.

NIOSH (National Institute for Occupational Safety and Health). 2001b. Rouge (Fe_2O_3), CAS 1309-37-1. NIOSH Pocket Guide to Chemical Hazards. September, 2001. Online at http://www.cdc.gov/niosh/npg/npgd0549.html.

NTP (National Toxicology Program). 2001a. Status Report for Ferric Chloride. Examined September, 2001. Online at: http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatf/7705080.Html.

NTP (National Toxicology Program). 2001b. NTP Chemical Repository Report for Ferric Chloride. Examined September, 2001. Online at: http://ntp-server.niehs.nih.gov/htdocs/CHEM_H&S/NTP_Chem7/Radian7705-08-0.html.

OSHA (Occupational Safety and Health Administration). 2001a. OSHA Preambles. Air Contaminants (29 CFR 1910). VI. Health Effects Discussion and Determination of Final PEL. Examined September, 2001. Online at http://www.osha-slc.gov/Preamble/AirCont_data/AIRCON6.html.

OSHA (Occupational Safety and Health Administration). 2001b. Iron Salts, Soluble (as Fe). Chemical Sampling Information. Revision dated January 15, 1993. Examined September, 2001. Online at http://www.osha-slc.gov/dts/chemicalsampling/data/CH_247600.html.

OSHA (Occupational Safety and Health Administration). 2001c. Iron Oxide Fume. Chemical Sampling Information. Revision dated January 11, 1999. Examined September, 2001. Online at http://www.osha-slc.gov/dts/chemicalsampling/data/CH_247400.html.

Stenbäck, F., J. Rowland and A. Sellakumar. 1976. Carcinogenicity of benzyo(a)pyrene and dusts in the hamster lung (instilled intratracheally with titanium oxide, aluminum oxide, carbon and ferric oxide. Oncology. 33: 29-34.

U.S. EPA. 1984. Health Effects Assessment for Iron (and Compounds). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. EPA 540/1-86-054.

U.S. EPA. 1991. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. April, 1991. OHEA-I-127.

U.S. EPA. 1994a. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. December, 1994. OHEA-I-127.

U.S. EPA. 1994b. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, Washington, D.C. October, 1994. EPA/600/8-90/066F.

U.S. EPA. 1995. Quarterly Status Report of RfD/RfC Work Group (as of 9/01/95). Office of Research and Development. National Center for Environmental Assessment, Cincinnati, OH.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. Annual Update. FY-1997. Prepared by the National Center for Environmental Assessment, Cincinnati, OH, for the Office of Research and Development, Office of Emergency and Remedial Response, Washington, D.C. July 1997. EPA-540-R-97-036. PB97-921199.

U.S. EPA. 2001. Substance index. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Examined September, 2001. Online at http://www.epa.gov/iris/subst/index.html.

WHO (World Health Organization). 2001. Online catalogs for the Environmental Health Criteria series. Examined September, 2001. Online at http://www.who.int/dsa/cat97/zehc.htm and http://www.who.int/dsa/justpub/add.htm.

7-29-05

Provisional Peer Reviewed Toxicity Values for

Iron and Compounds
(CASRN 7439-89-6)

Derivation of a Carcinogenicity Assessment

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	**provisional inhalation unit risk**
p-OSF	**provisional oral slope factor**
p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

7-29-05

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR IRON (CASRN 7439-89-6) AND COMPOUNDS
Derivation of a Carcinogenicity Assessment

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

A cancer assessment for iron is not listed on IRIS (U.S. EPA, 2005a), the HEAST (U.S. EPA, 1997), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2000), and was not considered by the CRAVE Work Group (U.S. EPA, 1995). The CARA list (1991, 1994) includes a Health Effects Assessment for Iron and Compounds (U.S. EPA, 1984) that assigned iron and its compounds to weight-of-evidence Group C, possible human carcinogen. This assessment was based on conflicting evidence of lung tumors following occupational inhalation exposure to ferric oxide (mixed exposure), and injection-site tumors in one patient and in mice treated with iron-dextran. IARC (1972, 1987) assigned ferric oxide to Group 3, not classifiable as to its carcinogenicity to humans based on inadequate data in humans (increased incidence of lung cancer following occupational exposure to iron dusts in mixtures) and apparently negative

evidence for carcinogenicity in mice, hamsters and guinea pigs exposed by inhalation or intratracheal instillation. For ferric oxide dust and fume, the ACGIH (1991, 2001) lists an A4 notation, not classifiable as a human carcinogen; this is based on mixed exposure studies in humans and primarily negative studies in animals. In March, 2004, a literature search was also conducted using TOXLINE, MEDLINE, Chemical Abstracts and Biological Abstracts data bases.

Iron has not been the subject of a toxicological review by ATSDR (2001) or the WHO (2001). Monographs by IARC (1972, 1984, 1987), a toxicity review on iron (Grimsley, 2001), and the NTP (2001a, 2001b) management status report and chemical repository summary were consulted for information relevant to the carcinogenicity of iron and inorganic iron compounds. The following computer searches, performed in April, 1993, were screened to identify additional pertinent studies not discussed in review documents: TOXLINE (1983-April, 1993), CANCERLIT (1990 - April, 1993), MEDLINE (1991 - April, 1993), TSCATS, RTECS, and HSDB. Update literature searches were conducted in September, 2001 in TOXLINE (1992-September, 2001), CANCERLIT (1992- September, 2001), MEDLINE (1992-September, 2001), TSCATS, RTECS, DART/ETICBACK, EMIC/EMICBACK, HSDB, GENETOX, and CCRIS.

REVIEW OF PERTINENT LITERATURE

Human Studies

Oral Exposure

Because iron is an essential element, the NAS (2001) has established guidelines for daily dietary intakes, based on gender, age, and physiological status, that are designed to avoid adverse effects of deficiency and excess. Individuals of northern European descent who are affected by hereditary hemochromatosis, an autosomal, recessive disorder, are not protected by these guidelines. These individuals exhibit excessive absorption of dietary iron, which results in abnormally high accumulations of iron in liver and brain tissues. When the liver consequently develops cirrhosis, the risk of developing primary hepatocellular carcinoma increases significantly. It is not clear whether these findings are relevant to excess iron intake by the general population.

Bird et al. (1996) investigated the association between plasma ferritin and iron intake and the development of adenomatous polyps, which are intermediate markers for colorectal cancer. The study population consisted of men and women between the ages of 50 and 75 years old who underwent routine screening by flexible sigmoidoscopy at one of two medical centers during 1991-1993. Individuals with cancer, inflammatory bowel disease, or familial polyposis were excluded. Cases (300 men and 167 women) were subjects diagnosed for the first time with one

or more histologically confirmed adenomatous polyps. Controls (331 men and 167 women) had no history of polyps and none discovered at sigmoidoscopy. Cases and controls were matched by sex, age (± 5 years), date of sigmoidoscopy (± 3 months), and medical center. Plasma ferritin levels, hematocrit, and certain nutritional indicators (carotenoids, ascobate, folate) were measured in blood samples drawn 6 months after examination. Iron intakes for the year preceding sigmoidoscopy were estimated by means of a semiquantitative food frequency questionnaire. After controlling for possible confounding factors, subjects with high plasma ferritin levels (>289 μg/L) had a multivariate-adjusted odds ratio for colorectal polyps of 1.5 (95% confidence interval (C.I. = 1.0-2.3) compared to subjects with low/normal levels (73-141 μg/L). The pattern for iron intake was U-shaped. Compared with subjects consuming an adequate amount of iron (11.6-13.6 mg/day), multivariate-adjusted odds ratios for colorectal polyps in men were 1.6 (95% C.I. = 1.1-2.4) for intakes below 11.6 mg/day and 1.4 (95% C.I.= 0.9-2.0) for intakes above 27.3 mg/day. The highest odds ratio of 2.1 (95% C.I. = 1.3-3.5) was found after further adjustment for smoking for men at the lowest level of iron intake. The association between iron intake and colorectal polyps disappeared when exposure group class of reaction was based on dietary intake alone (i.e., high iron supplementation ignored). The authors concluded that there was a weak positive association between iron exposure and colorectal polyps that may increase the risk of colorectal cancer but note that some factor in supplementation may have been responsible for the effect.

Inhalation Exposure

Most studies of cancer incidence following occupational exposure to iron dust are excluded from consideration because of confounding exposures to silica, radon daughters, soot, asbestos, or other types of metals in the study populations (U.S. EPA, 1984; IARC, 1972, 1984, 1987).

A case-control study examined cancer incidence in a Swedish male worker population (1958-1971) with a high exposure to iron oxides from the production of sulfuric acid from pyrite (FeS_2) (Axelson and Sjöberg, 1979). The workers were exposed to iron oxide (Fe_2O_3) along with 1-2% copper, 0.01-0.1% arsenic, nickel and cobalt as impurities. Exposure in the workroom was estimated as approximately 50-100 mg/m^3, and the particle size as 25% below 10 μm and 5-10% below 5 μm. No cases of siderosis were known from the plant. The Swedish National Cancer Register was consulted for locating cases of cancer that could have been caused by environmental exposure; the study examined cancers of the stomach, liver, lung, kidney, and bladder, and hematological malignancies. Each cancer case was matched with two controls from the local population register by matching for sex, age, and residency in the same or adjacent neighborhood block. Company files were searched to determine the length of exposure; those with less than 5 months of exposure were considered to be nonexposed. The study found no association between exposure to iron oxides and any of the selected types of cancer.

Animal Studies

Oral Exposure

Groups of F344 rats (50 per sex per group) were given ferric chloride ($FeCl_3 \cdot 6H_2O$) in drinking water at concentrations of 0, 0.25, or 0.5% (weight/volume) for 104 weeks, and then given distilled water for an 8 week recovery period (Sato et al., 1992). The intake of ferric chloride was reported to be 0, 169.7, or 319.7 mg/kg-day for males and 0, 187.9, or 336.0 mg/kg-day for females. The iron intakes were 0, 58.4, or 110 mg/kg-day in males and 0, 64.6, or 115.6 mg/kg-day in females. Rats were observed daily for clinical signs and mortality. Body weights were measured once a week for 13 weeks and every fourth week thereafter. All rats dying prematurely and survivors at week 112 were examined for gross and microscopic neoplastic and non-neoplastic lesions. There were dose-related decreases in drinking water intake and terminal body weight in both sexes. These may have been related to reduced palatability. Survival in both sexes was not significantly affected by exposure to ferric chloride. No increases in tumor incidence were observed in rats exposed to ferric chloride for two years.

Inhalation Exposure

Groups of male Syrian hamsters (132 per group) were exposed to filtered air or Fe_2O_3 (analytic grade) dust at a concentration of 40 mg/m^3, 6 hours/day, 5 days/week for life (Nettesheim et al., 1975). The particle size had a geometric mean diameter of 0.11 μm. In addition, two satellite groups (15 hamsters per treatment) were sacrificed, three animals at a time, at 2, 4, 8, 12, and 104 weeks, so that the accumulation of iron in the lung from inhaled Fe_2O_3 could be compared to background iron concentrations in heme. The animals were examined daily, before and after each exposure, for clinical signs; body weights were recorded monthly. All animals except those cannibalized (<2%) were necropsied. Histological analyses were performed for the major organs, including heart, trachea, lungs, and nasal cavities. Examination of the satellite groups demonstrated a gradual increase in iron accumulation in the lung, reaching a total of 10 mg per lung at 104 weeks. Exposure to Fe_2O_3 had no effect on survival or body weight gain and did not increase the incidence of tumors. The authors concluded that inhalation of Fe_2O_3 was not carcinogenic to hamsters.

Groups of Syrian golden hamsters (24 per sex per group) received intratracheal instillations of 0 or 3 mg[1] of Fe_2O_3 dust in 0.2 ml of saline once a week for 15 weeks, and then were observed up to week 120 (Stenbäck et al., 1976). Analysis by the sedimentation method demonstrated that 98% of the particles were less than 10 μm in diameter. Animals were weighed

[1]The authors characterized the treatment as a 'maximum dose of 3 mg'. It is not clear whether the hamsters received lower doses on some occasions.

weekly and autopsied. Organs with gross lesions and the larynx, trachea, bronchi, and lungs were examined histologically. Treatment with ferric oxide had no effect on survival and did not affect body weight except during the final weeks of survival (data not shown). Treatment did not induce tumors of the respiratory tract and the incidence of forestomach papillomas in the treatment group was less than in the control group.

Other Studies

Genotoxicity

Genotoxicity assays of inorganic iron salts were primarily negative in bacteria, but were more often positive in mammalian systems. Iron did not induce reverse mutations in *Salmonella typhimurium* strains TA98, TA102, TA1535, or TA1537, with or without activation (Wong, 1988). Ferric chloride and ferrous sulfate tested negative in strains TA98, TA100, TA1535, TA1537, and TA1538 with or without metabolic activation (Shimizu et al., 1985; Dunkel et al., 1999). Ferrous sulfate also tested negative in strains TA97 and TA102, with or without activation (Fujita et al., 1994), but positive in TA1537 and TA1538 (U.S. EPA, 1984). Ferrous and ferric chloride did not induce DNA repair in *Bacillus subtilis* (rec assay) (Leifer et al., 1981). Ferrous sulfate increased the frequency of mutations at the TK locus of mouse L5178Y lymphoma cells, with or without metabolic activation, but only at high concentrations that were likely to be cytotoxic; ferric chloride only increased the frequency of TK mutations when tested with metabolic activation (Dunkel et al., 1999). Ferrous sulfate did not induce sister chromatid exchanges *in vitro* (Ohno et al., 1982). DNA-protein cross-links were generated in mammalian cells cultured in the presence of ferrous iron (Altman et al., 1995). Single- and double-strand DNA breaks were produced in supercoiled plasmid DNA (Toyokuni and Sagripanti, 1992) and in isolated rat liver nuclei (U.S. EPA, 1984) treated with ferrous or ferric chloride. No breakage was detected electrophoretically in Chinese hamster ovary cell DNA treated with ferrous chloride (U.S. EPA, 1984). In a model of oxidative damage within cells, ferrous sulfate, in the presence of hydrogen peroxide, was demonstrated to induce double-strand breaks and intra-strand cross-links in DNA *in vitro* (Lloyd and Phillips, 1999).

Cell transformation

Iron compounds have yielded variable results in studies of cell transformation *in vitro*. Particles of magnetite (Fe_3O_4) induced transformation of cultured a Chinese hamster lung cell line (V_{79}), but only at cytotoxic concentrations (Elias et al., 1995). Ferrous chloride and ferrous sulfate induced cell transformation in viral-enhanced Syrian hamster embryo (SA7/SHE) cells (U.S. EPA, 1984).

Mechanistic Studies

Adverse effects of iron are thought to be related to the formation of reactive oxygen species via the Fenton reaction (Henle and Linn, 1997). Hydrogen peroxide can react with ferrous ion, resulting in the conversion to ferric ion and the production of hydroxyl radicals. Ferric ion can also react with hydrogen peroxide, producing superoxide radical. Reactive oxygen species may react with DNA. However, because of the complex homeostatic mechanisms involved in iron transport and metabolism, unbound ferrous iron is not likely to be present except in conditions of excessive iron intake.

PROVISIONAL WEIGHT-OF-EVIDENCE CLASSIFICATION

U.S. EPA (1984) classified iron and its compounds, including ferric dextran, as possible human carcinogens (Group C). This assessment was based on reports associating an increased incidence of lung cancer with exposure to hematite dust (confounded by coincident exposures to tobacco, alcohol, silica, soot, and fumes of other metals), inconsistent reports of lung tumors in animals exposed by inhalation or tracheal instillation to ferric oxide, and reports of injection site tumors in one patient injected with iron dextran and in mice injected with iron dextran or saccharated iron oxide. The current PPRTV assessment excludes organic forms of iron and studies in which the levels of impurities are significant.

Results of the case-control study by Bird et al. (1996) provide evidence of a weak association between elevated iron intake or high plasma ferritin (a measure of body stores) and the prevalence of adenomatous colorectal polyps, a possible precursor to colorectal cancer. Weaknesses of this study include the 6-month period between examination and ferritin measurements, and the possible recall errors affecting the dietary questionnaire for the previous year. In addition, the association between iron intake and colorectal polyps was stronger at low iron intake and not related to dietary (i.e., environmental) intake. Although the association between cirrhotic hereditary hemochromatosis and hepatocellular carcinoma is well established, the evidence for dietary iron intake and hepatic cancer in the general population was characterized by the NAS (2001) as inconclusive. In a chronic rat assay, Sato et al. (1992) found no evidence of carcinogenicity of ferric chloride ingested in drinking water at concentrations up to 0.5%. In summary, the evidence for carcinogenicity of ingested inorganic iron compounds in humans and animals is inadequate.

Evidence from the case-control study of Axelson and Sjöberg (1979) suggests that inhaled iron oxide may not be carcinogenic to humans. However, uncertainty remains because levels of exposure were not measured, the durations of exposure were not reported, and individuals exposed for up to 5 months were categorized as 'nonexposed.' In addition, the lack of reported cases of siderosis in the workplace suggests that the exposure levels may have been

lower than estimated. Thus, the evidence for carcinogenicity of inhaled iron oxide in humans is considered inadequate. Results of the study of Nettesheim et al. (1975) indicate that chronic inhalation exposure to iron oxide at a concentration of 40 mg/m^3 is not carcinogenic to hamsters. This finding is supported by the negative results for carcinogenicity of iron oxide administered by intratracheal instillation to hamsters for 15 weeks (Stenbäck et al., 1976). However, as both hamster studies used single exposure concentrations, the possibility of carcinogenicity at higher exposure levels cannot be disregarded.

Following the U.S. EPA (2005b) guidelines for carcinogen risk assessment, the available data are inadequate for an assessment of the human carcinogenic potential of inhaled iron oxide or ingested iron chloride.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Derivation of quantitative estimates of cancer risk for ingested or inhaled iron or iron oxide is precluded by the absence of adequate data demonstrating carcinogenicity.

REFERENCES

ACGIH (American Conference of Government Industrial Hygienists). 1991. Iron Oxide, CAS: 1309-37-1. Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. ACGIH. Cincinnati, OH. p. 803-805.

ACGIH (American Conference of Government Industrial Hygienists). 2001. Threshold limit values (TLV) for chemical substances and physical agents and biological exposure indices. ACGIH, Cincinnati, OH. p. 36.

Altman, S.A., T.H. Zastawny, L. Randers-Eichhorn et al. 1995. Formation of DNA-protein cross-links in cultured mammalian cells upon treatment with iron ions. Free Radic. Biol. Med. 19: 897-902.

ATSDR (Agency for Toxic Substances and Disease Registry). 2001. Internet HazDat - Toxicological Profile Query. Examined September, 2001. Online at http://www.atsdr.cdc.gov/gsql/toxprof.script.

Axelson, O. and A. Sjöberg. 1979. Cancer incidence and exposure to iron oxide dust. J. Occup. Med. 21: 419-422.

Bird, C.L., J.S. Witte, M.E. Swendseid, et al. 1996. Plasma ferritin, iron intake, and the risk of colorectal polyps. Am. J. Epidemiol. 144: 34-41.

Dunkel, V.C., R.H. San, H.E. Seifried and P. Whittaker. 1999. Genotoxicity of iron compounds in *Salmonella typhimurium* and L5178Y mouse lymphoma cells. Environ. Molec. Mutagen. 33: 28-41.

Elias, Z., O. Poirot, O. Schneider et al. 1995. Cytotoxic and transforming effects of some iron-containing minerals in Syrian hamster embryo cells. Cancer Detect. Prevent. 19: 405-414.

Fujita, H., N. Aoki and M. Sasaki. 1994. Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102. IX. Tokyo-Toritsu Eisei Kenkyusho Kenkyu Nenpo. 45: 191-199.

Grimsley, L.F. 2001. Iron and cobalt. In: Patty's Toxicology, 5th Edition, vol. 3, E. Bingham, B. Cohrssen and C.H. Powell, Eds. John Wiley and Sons, New York. p. 169-193.

Henle, E.S. and S. Linn. 1997. Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. J. Biol. Chem. 272: 19095-19098.

IARC (International Agency for Research on Cancer). 1972. Haematite and iron oxide. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 1, pp. 29-39. Lyon, France.

IARC (International Agency for Research on Cancer). 1984. Iron and steel founding. Polynuclear Aromatic Compounds, Part 3, Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 34, pp. 133-190. Lyon, France.

IARC (International Agency for Research on Cancer). 1987. Haematite and ferric oxide. Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs* Volumes 1 to 42. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Suppl. 7, pp. 216-219. Lyon, France.

Leifer, Z., T. Kada, M. Mandel et al. 1981. An evaluation oftests using DNA repair-deficient bacteria fro predicting genotoxicity and carcinogenicity. A report of the U.S. EPA's Gene-Tox program. Mutat. Res. 87: 211-297.

Lloyd, D.R. and D.H. Phillips. 1999. Oxidative DNA damage mediated by copper(II), iron(II) and nickel(II) Fenton reactions: evidence for site-specific mechanisms in the formation of

double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. Mutat. Res. 424: 23-36.

NAS (National Academy of Sciences). 2001. Iron. In: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Institute of Medicine, Food and Nutrition Board. National Academy Press, Washington, DC. p. 233-310. Online at: http://books.nap.edu/books/0309072794/html/.

Nettesheim, P., D.A. Creasia and T.J. Mitchell. 1975. Carcinogenic and cocarcinogenic effects of inhaled synthetic smog and ferric oxide particles. J. Natl. Cancer Inst. 55: 159-169.

NTP (National Toxicology Program). 2001a. Status Report for Ferric Chloride. Examined September, 2001. Online at: http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatf/7705080.Html.

NTP (National Toxicology Program). 2001b. NTP Chemical Repository Report for Ferric Chloride. Examined September, 2001. Online at: http://ntp-server.niehs.nih.gov/htdocs/CHEM_H&S/NTP_Chem7/Radian7705-08-0.html.

Ohno, H., F. Hanaoka and M.A. Yamada. 1982. Inducibility of sister-chromatid exchanges by heavy-metal ions. Mutat. Res. 104: 141-145.

Sato, M., F. Furukawa, K. Toyoda et al. 1992. Lack of carcinogenicity of ferric chloride in F344 rats. Food Chem. Toxicol. 30: 837-842.

Shimizu, H., Y. Suzuki, N. Takemura et al. 1985. Results of microbial mutation test for forty-three industrial chemicals. Sangyo Igaku. 27: 400-419.

Stenbäck, F., J. Rowland and A. Sellakumar. 1976. Carcinogenicity of benzyo(a)pyrene and dusts in the hamster lung (instilled intratracheally with titanium oxide, aluminum oxide, carbon and ferric oxide. Oncology. 33: 29-34.

Toyokuni, S. and Sagripanti, J.L. 1992. Iron-mediated DNA damage: sensitive detection of DNA strand breakage catalyzed by iron. J. Inorg. Biochem. 47: 241-248.

U.S. EPA. 1984. Health Effects Assessment for Iron (and Compounds). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. EPA 540/1-86-054.

U.S. EPA. 1991. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. April, 1991. OHEA-I-127.

U.S. EPA. 1994. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. December, 1994. OHEA-I-127.

U.S. EPA. 1995. Quarterly Status Report of RfD/RfC Work Group (as of 9/01/95). Office of Research and Development. National Center for Environmental Assessment, Cincinnati, OH.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. Annual Update. FY-1997. Prepared by the National Center for Environmental Assessment, Cincinnati, OH, for the Office of Research and Development, Office of Emergency and Remedial Response, Washington, D.C. July 1997. EPA-540-R-97-036. PB97-921199.

U.S. EPA. 2000. Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. Summer, 2000. EPA 822-B-00-001. Examined September, 2001. http://www.epa.gov/ost/drinking/standards/dwstandards.pdf

U.S. EPA. 2005a. Integrated Risk Information System (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Online. http://www.epa.gov/iris/

U.S. EPA. 2005b. Guidelines for Carcinogen Risk Assessment. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA/630/P-03/001F.

WHO (World Health Organization). 2001. Online catalogs for the Environmental Health Criteria series. Examined September, 2001. Online at http://www.who.int/dsa/cat97/zehc.htm and http://www.who.int/dsa/justpub/add.htm.

Wong, P.K. 1988. Mutagenicity of heavy metals. Bull. Environ. Contam. Toixicol. 40: 597-603.