TOXICOLOGICAL REVIEW

OF

TRIVALENT CHROMIUM

(CAS No. 16065-83-1)

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

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FOREWORD

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

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

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

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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

LIST OF ABBREVIATIONS

BAL	bronchoalveolar lavage
BMD	benchmark dose
BW	body weight
CASRN	Chemical Abstracts Service Registry Number
ESADDI	estimated safe and adequate daily dietary intake
GTF	glucose tolerance factor
IRIS	Integrated Risk Information System
MTD	maximum tolerated dose
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
ppb	parts per billion
ppm	parts per million
RfC	inhalation reference concentration
RfD	oral reference dose

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

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

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

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

memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

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

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Chromium (Cr) is a metallic element belonging to the first transitional series of the periodic table. Elemental chromium has a CAS Registry Number of 7440-47-3. The three most stable forms in which chromium occurs in the environment are the 0 (metal and alloys), +3 (trivalent chromium), and +6 (hexavelent chromium) valence states. In the +3 valence state, the chemistry of chromium is dominated by the formation of stable complexes with both organic and inorganic ligands (Hartford, 1979). In the +6 valence state, chromium exists as oxo species such as CrO_3 and CrO_4^{2-} that are strongly oxidizing (Cotton and Wilkinson, 1980).

Chromium in the ambient air occurs from natural sources, industrial and product uses, and burning of fossil fuels and wood. The most important industrial sources of chromium in the atmosphere originate from ferrochrome production. Ore refining, chemical and refractory processing, cement-producing plants, automobile brake lining and catalytic converters for automobiles, leather tanneries, and chrome pigments also contribute to the atmospheric burden of chromium (Fishbein, 1981). Chromate chemicals used as mist inhibitors in cooling towers and the mist formed during chrome plating are probably the primary sources of Cr(VI) emitted as mists in the atmosphere (Towill et al., 1978).

Scarce information exists in the literature regarding the nature of the chemical species present in the atmosphere. Under normal conditions, Cr(III) and Cr(0) in the air do not undergo any reaction (Towill et al., 1978). Cr(VI) in the air eventually reacts with dust particles or other pollutants to form Cr(III) (NAS, 1974); however, the exact nature of such atmospheric reactions has not been studied extensively. Chromium is removed from air by atmospheric fallout and precipitation (Fishbein, 1981). The atmospheric half-life for the physical removal mechanism depends on the particle size and particle density of atmospheric chromium. Chromium particles of small aerodynamic diameter (< 10 μm) may remain airborne for long periods and may be transported great distances by wind currents and diffusion forces.

Surface runoff, deposition from air, and release of municipal and industrial waste waters are the sources of chromium in surface waters. The most significant removal mechanism for Cr(III) from the aquatic environment is precipitation as $Cr_2O_3 \cdot \times H_2O$ followed by sedimentation. Cr(VI), however, can exist in aquatic media as a water-soluble complex anion and may persist in water for long periods. Cr(VI) is a moderately strong oxidizing agent and will react with organic

matter or other reducing agents to form Cr(III). Therefore, in surface water rich in organic content, Cr(VI) will exhibit a much shorter lifetime (Callahan et al., 1979).

Chromium probably occurs as insoluble $Cr_2O_3 \cdot \times H_2O$ in soil, given that organic matter in soil converts soluble chromate to insoluble Cr_2O_3 (U.S. EPA, 1983). There is no known chemical process that can cause chromium to be lost from soil. The primary processes by which chromium is lost from soil are physical. For example, chromium in soil can be transported to the atmosphere by way of dust or aerosol formation (U.S. EPA, 1983). Chromium is also transported from soil through runoff. Runoff can remove both chromium ions and bulk precipitates of chromium. In addition, flooding of soils and the subsequent anaerobic decomposition of plant matter may increase dissolution of Cr_2O_3 in soil through complexation (U.S. EPA, 1983). The water-soluble complexes may cause leaching of chromium from soil. Page (1981) reported the detection of a small concentration (l µg/L mean concentration) of chromium at a frequency of approximately 100% in ground water collected from New Jersey.

The bioconcentration factor (BCF) for Cr(VI) in fish muscle appears to be < l.0, but values of 125 and 192 were obtained for oyster and blue mussel, respectively (U.S. EPA, 1980). For Cr(III), BCF values of 116, 153, and 86 were obtained with the American oyster, soft shell clam, and blue mussel, respectively (U.S. EPA, 1983).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION FACTORS IN HUMANS AND EXPERIMENTAL ANIMALS

3.1.1. Oral

Based on fecal excretion of ^{51}Cr following oral administration of $^{51}CrCl_3$ to human patients, Donaldson and Barreras (1966) estimated absorption to be approximately 0.4%. When $^{51}CrCl_3$ was administered intraduodenally, absorption was not appreciably changed. In rats, approximately 2% of the intragastric dose of $CrCl_3$ appeared to be absorbed based on fecal excretion of chromium. Jejunal administration only slightly increased the apparent absorption of $CrCl_3$. Anderson et al. (1983) confirmed the low absorption of trivalent chromium in humans following the administration of 200 µg of Cr(III) trichloride, and they suggested that the absorption efficiency of trivalent chromium is dependent on dietary intake. Anderson et al. (1986) reported that at low levels of dietary intake (10 µg) about 2% of trivalent chromium was absorbed. When intake increases to > 40 µg, the absorption efficiency dropped to approximately 0.5%. Bunker et al. (1984) determined that elderly subjects absorbed less than 3% of trivalent chromium ingested in the diet.

A number of animal studies confirm that trivalent chromium is poorly absorbed in the gastrointestinal tract. Visek et al. (1953) estimated that less than 0.5% of ingested $CrCl_3$ was absorbed through the gastrointestinal tract of the rat. Mertz et al. (1965) estimated that rats absorbed less than 3% of a single dose of $CrCl_3$ by gavage. MacKenzie et al. (1959) estimated that less than 3% of a single dose of $CrCl_3$ by stomach tube was absorbed in rats. Ogawa (1976)

found gastrointestinal absorption of $CrCl_3$ to be less than 3% in rats. Henderson et al. (1979) determined that hamsters absorbed less than 1.5% of an administered oral dose of trivalent chromium. Furthermore, Mertz et al. (1965) reported that absorption in rats was independent of the administered dose and dietary chromium status (deficient or supplemented in chromium) of the animals. Cr(III) was found to be better absorbed in fasted than in fed rats (MacKenzie et al., 1959).

3.1.2. Inhalation

A number of factors can influence the absorption of chromium following inhalation, including the size, oxidation state and solubility of the chromium particles, the activity of alveolar macrophages, and the interaction of chromium with biomolecules following deposition in the lung (ATSDR, 1993). Absorption of inhaled chromium compounds following occupational exposure has been demonstrated by the measurement of chromium in the serum and urine and hair of workers in the chromium industry (Minoia and Cavalleri, 1988; Randall and Gibson, 1987; Tossavainen et al., 1980). Cr(III) is less well absorbed than Cr(VI) due to the relative inability of Cr(III) to cross cell membranes. However, workers exposed to Cr(III) lignosulfonate dust at 0.005-0.23 mg Cr(III)/m^3 had detectable concentrations of chromium in the urine at the end of the workday (Kiilunen et al., 1983).

Animal studies have shown that trivalent chromium is absorbed very slowly by inhalation. Baetjer et al. (1959a) administered $CrCl_3$ to guinea pigs intratracheally. Ten minutes post-treatment, 69% of the administered dose remained in the lungs, while 4% was found in the blood and tissues. Percentages of administered chromium found in the lungs 24 hours, 30 days, and 60 days post-treatment were 45%, 30%, and 12%, respectively. These investigators hypothesized that the slow absorption of trivalent chromium is due to the fact that it forms insoluble complexes with macromolecules. Furthermore, clearance from the respiratory tract to the gastrointestinal tract may be a factor when chromium compounds are administered by inhalation. Visek et al. (1953) found similar results when $^{51}CrCl_3$ was instilled intratracheally in guinea pigs. In this study, absorption from the lungs was estimated to be approximately 5%. The authors suggested that the majority of the $CrCl_3$ was cleared from the lungs by mucociliary action and passed through the gastrointestinal tract because 55% and 7% of the administered ^{51}Cr had been recovered from the feces and urine, respectively, within 7 days.

Wada et al. (1983) exposed male Sprague-Dawley strain rats to $CrCl_3$ at an atmospheric concentration of 14.1 mg/m^3 (Cr) and observed that the chromium was associated with both high- and low-molecular-weight proteins. The chromium that remained in the lungs was associated with the high-molecular-weight fraction, and this fraction slowly decreased with time following exposure. The level of chromium associated with the low-molecular-weight fraction remained constant for the 5 days of observation following treatment; however, chromium associated with this fraction accumulated with time in the liver. The authors suggested that the low-molecular-weight protein may be involved in the absorption and transport of chromium following inhalation.

Suzuki et al. (1984) exposed rats to potassium dichromate(VI) or Cr(III) trichloride by inhalation and determined that while lung clearance of both valence states was dependent on particle size, Cr(VI) was absorbed with threefold greater efficiency than Cr(III).

3.1.3. Distribution

Much work has been performed on in vivo reduction of Cr(VI) to Cr(III), and some characterizations can be made. Ingested hexavalent chromium is efficiently reduced to the trivalent form in the gastrointestinal tract (DeFlora et al., 1987). In the lungs, hexavalent chromium can be reduced to the trivalent form by ascorbate and glutathione. The reduction by ascorbate is more rapid than that by glutathione and results in a shorter residence time in the lungs (Suzuki and Fukuda, 1990). There is no evidence that trivalent chromium is converted to hexavalent chromium in biological systems (Amdur et al., 1993).

Once absorbed, Cr(III) compounds are cleared rapidly from the blood and more slowly from the tissues. Hopkins (1965) injected 0.1 µg ^{51}Cr (as chromium chloride)/100g intravenously in male rats. The blood chromium content as a percentage of the blood concentration decreased from 94% at 30 minutes to 17% in 24 hours and to 5% at 96 hours. Lim et al. (1983) followed distribution of $^{51}CrCl_3$ after intravenous administration in six adults. Within several hours of dosing, > 50% of the chromium in plasma was distributed to the liver, spleen, and other organs. After 3 months, the liver contained > 50% of the total body burden of ^{51}Cr.

Visek et al. (1953) reported organ distribution of several chromium salts following intravenous injection in rats. $CrCl_3$ concentrated in the liver, spleen, and bone marrow; once deposited, it cleared slowly. The liver in the $CrCl_3$-exposed rats was the only organ to clear significant amounts of chromium over the study period (45 days). In rats receiving intraperitoneal administration of Cr(III) nitrate for 30 or 60 days, the highest levels of chromium were observed in the liver, followed by the kidneys, testes, and brain. Tissue concentration increased nonlinearly with dose, and concentrations in the kidney increased significantly with duration (Tandon et al., 1979). Trivalent chromium was detected only in the livers of mice following administration of 4.8, 6.1, or 12.3 mg Cr(III)/kg-day as chromium (III) trichloride in drinking water for 1 year (Maruyama, 1982). Tissue concentrations were 40-90 times below those reported following administration of hexavalent chromium in this study. MacKenzie et al. (1958) reported that tissue concentrations of rats given Cr(III) trichloride were ninefold lower than those given potassium dichromate in drinking water. Sullivan et al. (1984) treated adult and neonatal rats with an acute oral dose of radiolabeled Cr(III) trichloride. Seven days following the dose, neonates and adults retained approximately 35% and 0.2% of the dose in the gut, respectively. Neonates accumulated 0.12%, 0.05%, and 0.0088% in the kidney, liver, and lung, while adults accumulated 0.003%, 0.002% and 0.0003% in the kidney, liver and lung, respectively. Red blood cells were found to accumulate significantly more chromium than white blood cells following intravenous administration of $CrCl_3$ in the rat (Coogan et al., 1991).

Mice given a single intraperitoneal injection of Cr(III) trichloride were found to have retained 87%, 73%, and 45% of the dose on day 3, 7, and 21 post-treatment. The retention of

chromium was attributed to the formation of trivalent chromium complexes with proteins and amino acids (Bryson and Goodall, 1983).

Mertz (1969) studied placental transfer of a variety of chemical forms of trivalent chromium in rats. Male and female rats fed commercial or chromium-depleted yeast diets and drinking water with or without 2 ppb chromium were mated. Neonates whose dams were fed the commercial diet contained nearly twice the chromium as neonates of dams fed the chromium-deficient diet. Exposure of dams to trivalent chromium in drinking water did not increase the chromium content of the neonates. Neonatal concentrations of chromium were not increased following administration of $CrCl_3$ intravenously or by gavage before, during, or after mating. However, administration of chromium in the form of glucose tolerance factor (GTF) by gavage during gestation resulted in levels in neonates that were 20%-50% of those in the dams. Danielsson et al. (1982) studied placental transfer of trivalent chromium in mice following intravenous injection of $CrCl_3$. The highest maternal chromium concentrations were found in the renal cortex, skeleton, liver, and ovaries. The fetal concentration of chromium was 0.4% and 0.8% of the maternal serum concentration when dams were injected in mid- and late-gestation, respectively. Chromium accumulated in the fetal skeleton and yolk sac placenta. Iijima et al. (1983) also reported that trivalent chromium crossed the placenta of mice injected intraperitoneally with $CrCl_3$. Visek et al. (1953) reported that an insignificant amount of ^{51}Cr crossed the placenta of rats in the 24 hours following intravenous injection regardless of the chemical form injected, the valence state, the gestational stage, or the size of the litter. In no instance was the radioactivity measured in the fetuses greater than 0.13% of the dose. Casey and Hembridge (1984) demonstrated that chromium can be transferred to infants through breast milk. The breast milk of 45 lactating women was found to have a chromium content averaging 0.3 µg/L. These concentrations were taken to represent background levels in women whose chromium exposure occurs primarily through the diet.

Hexavalent chromium readily enters cells through the phosphate and sulfate anion-exchange carrier pathway, although a portion may remain in plasma for an extended period (Wiegand et al., 1985). While Cr(III) compounds are unable to cross the cell membrane by this pathway (Gray and Sterling, 1950), they may enter cells, but only with very low efficiency (Lewalter et al., 1985; O'Flaherty, 1996). Hexavalent chromium is reduced to the trivalent form intracellularly by the action of glutathione (Debetto and Luciani, 1988; Petrilli and De Flora, 1978b). Following reduction to the trivalent form, chromium may interact with cellular macromolecules, including DNA (Wiegand et al., 1985), or may be slowly released from the cell (Bishop and Surgenor, 1964).

A physiologically based model for chromium has recently been developed, which incorporates absorption and disposition schemes for Cr(VI) and Cr(III) throughout the body (O'Flaherty, 1996). The model was calibrated on the basis of published oral and intratracheal kinetic studies using soluble Cr(III) and Cr(VI) in the rat, and it accounts for most of the major features of chromium kinetics in the rat, including reduction of Cr(VI) to Cr(III). The model suggests the following in vivo disposition for chromium. Both Cr(III) and Cr(VI) are poorly absorbed from the lung and the gastrointestinal tract. Following inhalation exposure, chromium may be absorbed into the systemic circulation, transferred to the gastrointestinal tract by

mucociliary action, or remain in the lung. Cr(VI) is reduced to Cr(III) in all tissues, including the lung and the gastrointestinal tract. Both Cr(III) and Cr(VI) are better absorbed from the gastrointestinal tract in the fasted than in the fed state, and the absorption efficiency of Cr(III) salts is largely dependent on the nutritional status of the animal as well as the nature of the anion making up the Cr(III) salt. The model assumes that reduction of Cr(VI) does not occur in the plasma. Cr(VI) enters cells by the phosphate and sulfate anion-exchange carrier pathway. Cr(III) travels in the bloodstream largely bound to amino acids, other organic acids, and plasma proteins such as globulins. The complexes of Cr(III) which are bound to lower molecular weight ligands are most likely to be able to traverse cell membranes (Mertz, 1969). A significant amount of absorbed chromium is taken up in the bone (Witmer and Harris, 1991; Weber, 1983). Chromium is also concentrated in tissues of the liver, kidney, and spleen. Once in the cell, Cr(VI) may be reduced to Cr(III), which may subsequently interact with cellular macromolecules, including DNA (Wiegand et al., 1985), or may be slowly released from the cell (Bishop and Surgenor, 1964).

The model suggests that the bioaccessibility of chromium to absorption processes may be the single most important factor determining the toxicity of a specific chromium source (O'Flaherty, 1996).

Given the rapid reduction of Cr(VI) to Cr(III) in vivo, it is relevant to consider whether environmental exposures to Cr(VI) or administration of Cr(VI) in controlled animal experiments is essentially identical to environmental exposures to Cr(III) or administration of Cr(III) in controlled experiments. While considerably more data are available for Cr(VI) than for Cr(III), it appears at present that exposures to Cr(VI) have considerably different outcomes than exposures to Cr(III). Cr(VI) has been shown to be more toxicologically active than Cr(III) as it more readily crosses cell membranes. The Agency has prepared the Toxicological Reviews and IRIS Summaries for Cr(VI) and Cr(III) from this perspective.

3.1.4. Metabolism

Trivalent chromium potentiates the activity of insulin in vitro and in vitro. In the biologically active form, chromium occurs in a complex referred to as GTF, tentatively identified as a chromium-nicotinic acid complex. GTF has been suggested to operate through activation of membrane phosphotyrosine phosphatase in mammals, although the complete structure of the complex has not been identified (Mertz, 1993; Davis et al., 1996).

3.1.5. The Essentiality of Chromium

Cr(III) potentiates insulin action in peripheral tissue and is essential for lipid, protein, and fat metabolism in animals and humans. Chromium deficiency causes changes in the metabolism of glucose and lipids and may be associated with maturity-onset diabetes, cardiovascular diseases, and nervous system disorders (Anderson, 1993, 1995). The National Research Council (NRC) has identified an estimated safe and adequate daily dietary intake (ESADDI) for chromium of 50-200 µg/day (NRC, 1989), corresponding to 0.71-2.9 µg/kg-day for a 70 kg adult.

The Food and Drug Administration (FDA) has selected a Reference Daily Intake for chromium of 120 µg/d (U.S. DHHS, 1995).

4. HAZARD IDENTIFICATION

Hexavalent chromium is widely considered to have significantly greater toxicity than the trivalent form. This results in part from the recognition of hexavalent chromium as a known human carcinogen by the inhalation route of exposure, from the caustic properties of many of the hexavalent compounds, the greater absorption of the hexavalent species following exposure by ingestion and inhalation, and the ability of hexavalent chromium to efficiently traverse cell membranes. However, relatively few studies are available in the literature that directly address the toxicity of trivalent chromium, particularly by the inhalation route of exposure. This lack of data results in considerable uncertainty regarding the hazard associated with exposures to trivalent chromium.

4.1. STUDIES IN HUMANS

4.1.1. Oral

The essential role of trivalent chromium in glucose and lipid metabolism has been widely studied; however, only one study was located that addressed the oral toxicity of trivalent chromium in humans. Kusiak et al. (1993) reported increased mortality due to stomach cancer in gold miners in Ontario, Canada. Exposures to arsenic, chromium, mineral fiber, diesel emissions, and aluminum powder were considered as possible explanations for the excess stomach cancer. The authors found that the excess incidence of stomach cancer was best associated with the time-weighted index of exposure to chromium in miners under the age of 60. However, a similar association between the index of exposure to chromium and excess stomach cancer was not seen in older gold miners in this study. Although diet is an important risk factor in the onset of stomach cancer, the study was unable to consider the role of dietary habits in the onset of stomach cancer in the study population. While the authors suggest that chromium or a substance closely associated with chromium may be the causative agent for stomach cancer, the inability to consider important confounding factors and the absence of a clear pattern of disease incidence with increasing exposure make this association highly uncertain. The substantial uncertainties related to the association between chromium exposure and disease incidence and the significant confounding factors make this study unusable for risk assessment purposes.

4.1.2. Inhalation

Occupational exposure to chromium by inhalation has been studied in the chromate manufacturing and ferrochromium industries; however, exposures all include mixed exposures to both Cr(III) and Cr(VI). The Cr(VI) species is widely considered to be the etiologic agent in reports of excess cancer risk in chromium workers. However, studies are inadequate to rule out a

contribution by Cr(III), and Cr(VI) cannot be unequivocally demonstrated to be the etiologic agent for noncarcinogenic effects following inhalation.

Studies addressing exposures to Cr(III) alone are not available, and the role of Cr(III) in disease following exposures to mixtures of Cr(III) and Cr(VI) cannot be determined. Significant reduction of Cr(VI) to Cr(III) occurs in the lungs, and absorption of Cr(III) from lung tissue is known to occur (O'Flaherty, 1996). In order to be comprehensive, a summary of the results of studies involving mixed exposures to Cr(III) and Cr(VI) is presented below.

A number of epidemiologic studies have considered the association between inhalation of chromium and noncarcinogenic endpoints, including upper respiratory irritation and atrophy, lower respiratory effects, and systemic effects.

Bloomfield and Blum (l928) examined 23 men from six chromium plating plants in the United States. Fourteen of these workers typically spent 2-7 hours/day over vats of chromic acid, which generated airborne hexavalent chromium ranging from 0.12-5.6 mg/m^3. These men experienced nasal tissue damage, including perforated septum (2), ulcerated septum (3), chrome holes (6), nosebleed (9), and inflamed mucosa (9). In general, the nine remaining workers examined, who were not directly exposed to chromium vapors, had only inflamed mucosae. The authors concluded that chromic acid at concentrations greater than 0.1 mg/m^3 is likely to cause nasal tissue injury. However, while no concentrations lower than 0.12 mg/m^3 were observed, injury to nasal tissue caused by lower concentrations could not be ruled out.

Machle and Gregorius (1948) reported an incidence of nasal septal perforation of 43.5% in 354 employees who worked in a chromate-producing plant that manufactured sodium chromate and bichromate. At the time of the study, airborne chromate concentrations ranged from 0.1 to 2.8 mg/m^3. The plant had been in operation for at least 17 years, and some employees probably worked in the plant when reverberatory furnaces, a prominent source of high chromate exposure, were used.

Mancuso (1951) reported on physical examinations of a random sample of 97 workers from a chromate-chemical plant. The results indicated that 61 of the 97 workers (63%) had septal perforation. The data suggested to the author that Cr(III) may be partly responsible for the perforations; however, there were insufficient data to make an unequivocal conclusion.

The U.S. Public Health Service conducted a study of workers in seven chromate-producing plants in the early 1950s. Of 897 chromate industry workers in the study, 57% were found to have a nasal septum perforation. Perforated septum was observed even in workers employed fewer than 6 months. The study indicated that exposure to chromate results in severe nasal tissue destruction, but exposure levels were not measured; hence, the data are of limited usefulness for risk assessment purposes (Federal Security Agency, 1953).

Vigliani and Zurlo (1955) reported nasal septal perforation in workers exposed to chromic acid and chromates in concentrations of 0.11-0.15 mg/m^3. The lengths of exposure were not known. Hanslian et al. (1967) reported on otolaryngologic examinations of 77 persons

exposed to chromic acid aerosol during chrome plating. Of those, 19% were observed to have septal perforation and 48% to have nasal mucosal irritation. The workers averaged 6.6 years of exposure to an airborne chromium concentration of 0.4 mg/m^3. In 14 persons, papillomas of the oral cavity and larynx were found. The diagnosis of papilloma was confirmed by histologic examination. There were no signs of atypical growth or malignant degeneration.

Kleinfeld and Russo (1965) reported some degree of nasal septal ulceration in seven of nine workers in a chrome-plating plant, with four of seven demonstrating frank perforations. Analyses of air samples showed chromium concentrations of 0.18-1.4 mg/m^3. Data regarding the length of exposure and exposure concentration for individual workers were not available.

Gomes (1972) examined 303 employees who worked in 81 electroplating operations in Sao Paulo, Brazil. Over two-thirds of the workers had mucous membrane or cutaneous lesions, with many of them having ulcerated or perforated nasal septa. The duration of exposure was not stated, but the author mentioned that the harmful effects were noted in under 1 year. A direct correlation between workers exposed to a given airborne concentration of Cr(VI) and the development of harmful effects could not be made.

Cohen and Kramkowski (1973) and Cohen et al. (1974) examined 37 workers (7 male and 30 female) employed in the nickel-chrome department of an electroplating plant in comparison with 21 workers (15 male and 6 female) in other areas of the plant not significantly exposed to chromic acid. Smoking demographic data was not provided. Environmental air samples were collected from breathing zones of several workers in the exposed and control groups to determine concentrations of total chrome and Cr(VI). Brief medical histories were confined to the ear, nose, throat, and cutaneous structures. Within 1 year of employment, 12 workers experienced nasal ulceration or perforation. Nasal ulcers and perforations were associated with total chromium concentrations of 1.4 to 49.3 µg/m^3, averaging 7.1 µg/m^3, and Cr(VI) concentrations of 0.09 to 9.1 µg/m^3, averaging 2.9 µg/m^3. Ninety-five percent of the 37 workers studied exhibited pathologic changes in nasal mucosa in a concentration-duration response. More than half of the workers employed less than 1 year had nasal pathology that was more severe than simple redness of the nasal mucosa. Almost all the workers (35 of 37) employed longer than 1 year had nasal tissue damage. The authors noted the lack of good industrial hygiene practices, implicating direct contact, such as touching of the nose with chromium-contaminated hands, as a potentially important route of exposure.

Lucas and Kramkowski (1975) conducted a health hazard evaluation of 11 employees in the “hard” chrome area of an industrial plating facility. The average age of the employees was 39 years, and the average duration of employment in the hard chrome area was 7.5 years. Medical examinations were conducted to evaluate the presence of dermatitis, chrome holes, old chrome hole scars, ulcerated nasal septum, infection of the mucosa, nasal redness, perforated nasal septum, reddened throat, conjunctivitis, and wheezing. Environmental air samples were collected from the breathing zone on all workers in the hard chrome area to determine the concentrations of hexavalent chromium. Cr(VI) concentrations ranged from 1 to 20 µg/m^3, averaging 4 µg/m^3. However, the authors attributed the nasal pathology primarily to direct contact. Clinical observations included injection of the nasal mucosa in five workers, ulcerated

nasal septum in two workers, atrophic scarring indicative of the presence of past ulceration in two workers, and complete perforation of the nasal septum in four workers. Poor hygiene practices, including touching the nose with the hand, were noted at the plant and represented a confounding factor in the etiology of the nasal lesions.

Markel and Lucas (1973) conducted a health hazard evaluation of 32 workers at a "cold dip" chrome plating plant who were employed in the chrome department or who regularly spent a portion of their workday in that area. Twenty of the employees worked in the chrome area of the plant for more than 5 years. A total of 16 personal and 7 general air samples were taken to determine the concentrations of Cr(VI). Maximum airborne Cr(VI) concentration was 3 $\mu g/m^3$. No workers were found to have ulcerated nasal mucosa or perforated nasal septa. Half of the 32 employees had varying degrees of mucosal irritation. The authors did not consider this to be significant, because the survey was carried out at the peak of the 1972-1973 influenza epidemic.

Lindberg and Hedenstierna (1983) compared lung function, the condition of the nasal septum, and subjective symptoms related to respiratory health (data obtained by questionnaire) in unexposed controls (119) and workers (43) exposed to chromic acid in chrome plating operations. Workers were further divided into low (< 2 μg Cr[VI]/m^3) and high (> 2 μg Cr[VI]/m^3) exposure groups. Complaints of diffuse nasal symptoms ("constantly running nose," "stuffy nose," or "a lot to blow out") were registered by 4/19 workers in the low group and half of the 24 workers in the high group. The authors reported reddening of the nasal mucosa at 1 to 2 $\mu g/m^3$ and nasal irritation (chronic and nasal septal ulceration and perforation) in two-thirds of the subjects at concentrations from 2 to 20 $\mu g/m^3$. All workers with nasal ulceration had been exposed to chrome acid mist, which contained Cr(VI) at 20 $\mu g/m^3$, or greater near the baths. For pulmonary function measurements, changes in vital capacity and forced expiratory volume at 1 second (FEV_1) were seen from Cr(VI) exposures greater than 2 $\mu g/m^3$. Examination of the nasal septum revealed that damage was significantly greater in exposed workers than in unexposed controls and appeared to be somewhat more severe in the high group than in the low group. There was a tendency for lung function parameters to return to normal over a 2-day weekend.

In the United States, 97 workers in chromate-producing plants had a higher incidence of severely red throats and pneumonia, but they did not show any increase in the incidence of other respiratory diseases when compared with control groups. Although bilateral hilar enlargement was observed, there was no evidence of excessive pulmonary fibrosis in these workers (Federal Security Agency, 1953). The various lung changes described in these workers may represent a nonspecific reaction to irritating material or a specific reaction to chromium compounds. Many of the conditions mentioned occur widely in the general population (NAS, 1974).

Lindberg and Vesterberg (1983b) studied urinary excretion of proteins in 24 currently employed chrome platers and 27 former chrome platers. Results were compared with those for a group of 37 referents. Exposures for current workers were determined using personal samplers and were found to range from 2 to 20 $\mu g/m^3$, with an average level of 6 $\mu g/m^3$. Exposures of former platers were thought to be higher than those for the current workers. The duration of exposure ranged from < 1 to 26 years. Cr(VI) exposure was found to result in renal effects in a dose-dependent fashion (based on elevated excretion of β-2-microglobulin as an indicator of

nephrotoxicity) in current workers exposed to 4 to 20 μg/m^3 Cr(VI) over 8-hour shifts. The effect may be reversible since former chrome platers did not have an elevated concentration of either B-2-microglobulin or albumin in their urine. Most of the currently exposed workers were also observed to have irritation symptoms of the airways, including ulcerated nasal septum and complete perforations. Severe objective and subjective levels for the airway effects occurred at no-observed-adverse-effect levels (NOAELs) for renal toxicity.

In another study, Saner et al. (1984) did not find increased urinary β-2-microglobulin levels in tannery workers in comparison with referent control workers. However, comparison of urinary chromium concentrations of the tannery workers in this study versus the chrome platers in the Lindberg and Vesterberg (1983a,b) study suggests that the latter had distinctly higher chromium exposures.

Exposure to vapors of chromium salts has been suspected as a cause of asthma, coughing, wheezing, and other respiratory distress in ferrochromium workers (Langard, 1980). Novey et al. (1983) identified chromium-specific antibodies in a 32-year-old white male worker who experienced a productive cough, wheezing, and dyspnea within 2 weeks of beginning a new job electroplating with chromium. Laboratory testing of this individual was performed with placebo and nickel and chromium solutions vaporized by heat. The nickel and chromium solutions precipitated asthmatic symptoms identical to those experienced on the job. The authors concluded that the affected individual developed an acquired sensitivity to chromium and nickel vapors.

Actual Cr(III) and Cr(VI) exposure levels in many of the studies attributing respiratory effects to chromium were unknown. In addition, data on other confounding factors such as smoking were frequently unavailable. These caveats significantly complicate determination of the potential health effects associated with exposure to chromium.

Various other disease states have been attributed to chromium, but in most cases, the etiologic relation to chromium is doubtful because of the presence of other chemicals (NAS, 1974). These studies, reviewed by the U.S. EPA (1984), will not be reviewed here.

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

4.2.1. Chronic Oral Studies

Two oral chronic rodent bioassays of Cr(III) were located in the literature. Ivankovic and Preussman (1975) fed 60 male and female rats (per dose group) 0%, l%, 2%, or 5% Cr_2O_3, baked in bread, 5 days/week for 600 feeding days (120 weeks). The primary purpose of this study was to assess the carcinogenic potential of Cr_2O_3. The authors estimated, based on measures of food consumption and body weight (bw), that rats consumed 360 g/kg bw, 720 g/kg bw, and 1,800 g/kg bw of total Cr_2O_3 over the duration of the study in the l%, 2%, and 5% Cr_2O_3 feeding groups, respectively. The animals were maintained on control diets following termination of the

exposure until they became moribund or died. No adverse effects were noted at any feeding level. The highest dose level (5%), which represented a total Cr_2O_3 consumption for 600 days of feeding of 1,800 g/kg bw, corresponds to a total Cr(III) intake of 1,232 g/kg or 1,467 mg/kg-day, expanding exposure over 840 days (600 days at 5 days/week = 120 weeks or 840 days). The lack of toxicity given the high concentrations of trivalent chromium used in the dose groups may reflect the poor absorption of trivalent chromium by the oral route of exposure (Visek et al., 1953; Mertz et al., 1965; MacKenzie et al., 1959; Ogawa, 1976; Henderson et al., 1979). Cr(III) has been found to be better absorbed in fasted than in fed rats (MacKenzie et al., 1959). The use of baked bread as a vehicle for dosing may have further reduced the absorption of chromium in the Ivankovic and Preussman (1975) study.

Schroeder et al. (1965) exposed 54 male and 54 female Swiss mice to drinking water that contained 5 ppm chromium (as chromium acetate) for life (0.46 mg Cr(III)/kg/day). No increase in the incidence of tumors was seen in the treated animals with respect to controls. Similar results were obtained by Schroeder et al. (1965) for Long-Evans rats. The dose of trivalent chromium used in this study was 2,000- to 10,000-fold lower than the dose in the Ivankovic and Preussman (1975) study, and most likely did not approach the maximum tolerated dose (MTD). The Schroeder et al. (1965) study is considered to be an inadequate test of carcinogenicity.

4.2.2. Subchronic Oral Studies

Several subchronic studies regarding oral exposure to trivalent chromium were located in the literature. Akatsuka and Fairhall (1934) fed cats 50-100 mg of Cr(III)/day for 1-3 months. No effects on weights or gross or microscopic pathology of major organs were noted. This study cannot be used for quantitative risk assessment since the dose and duration of exposure were not defined precisely.

MacKenzie et al. (1958) provided rats with 25 ppm Cr(III) in drinking water for 12 months and noted no change in body weight, macroscopic or microscopic pathology, or clinical chemistry variables. The MacKenzie et al. (1958) study suggests a no-observed-effect level (NOEL) at 25 ppm $CrCl_3$, equivalent to 8.2 ppm trivalent chromium. Assuming that an average rat weighs 0.35 kg and consumes 0.035 L water/day, 8.2 ppm is adjusted to 0.82 mg trivalent chromium/kg bw/day.

Ivankovic and Preussman (1975) fed rats baked bread containing up to 5% chromic oxide (Cr_2O_3) for 90 days. The only effects observed were reductions in the absolute weights of the livers and spleens of animals in the high-dose group, which does not necessarily represent an adverse effect. This study suggests a NOAEL of 5% Cr_2O_3 (50,000 ppm), although no clear adverse effects were observed at any dose used in the study. The authors calculated, based on measured food consumption and body weight, that male rats in the 5% feeding group consumed 180 g/kg Cr_2O_3 total over the 88-day experimental period. This corresponds to 1,399 mg/kg-day Cr(III).

Anderson et al. (1997) fed Sprague-Dawley rats 0-100 mg/kg Cr(III) chloride or Cr(III) tripicolinate (trivalent chromium coordinated with 2-carboxypyridine) in the diet for 24 weeks.

No statistical differences in body weight or blood variables were noted among the groups examined at 11, 17, or 24 weeks. Histological examination of the animals in the high-dose groups did not reveal any detectable differences. Animals fed chromium picolinate were found to have liver and kidney chromium concentrations two- to threefold greater than those fed chromium chloride, demonstrating the higher absorption of chromium tripicolinate. No toxicity was observed in any of the dose groups in this study.

4.2.3. Chronic Inhalation Studies

Several animal studies have been performed to assess the carcinogenic potential of Cr(III) in the respiratory tract. Only one study was located that utilized an exposure route similar to that expected for humans (inhalation of chromium dusts) (Baetjer et al., 1959b).

Three strains of mice (strain A, Swiss, and C57BL) were exposed to chromium-containing dust (Baetjer et al., 1959b). These strains have, respectively, high, medium, and low spontaneous lung tumor incidences. The dust was similar to that found in the chromium chemical manufacturing industry, containing 13.7% Cr(VI) oxide (CrO_3) and 6.9% Cr(III) oxide (Cr_2O_3), along with other metal oxides. In addition, potassium dichromate ($K_2Cr_2O_7$) was added at a level of 1%. The animals were exposed to the dust-laden atmosphere containing between 0.5 and 1 mg total chromium 4 hours/day, 5 days/week for an average of 39.7 weeks (range of 16 to 58 weeks). At death or termination of exposure, the lungs were examined by means of a low-power microscope, and abnormal tissues were submitted for histologic confirmation of tumors.

The incidence of lung tumors was not different in exposed mice of any strain as compared with approximately equal numbers of the appropriate controls of unexposed mice of the same age. There was also no difference in those strains having high spontaneous tumor incidence with regard to the average number of tumors per mouse or the percent of mice with multiple tumors. The lung tumors present in both control and treated animals were adenomas, which appeared to be histologically similar; however, in exposed animals, the adenomas developed slightly earlier in the strain A mice. Three additional small groups of mice (two groups of 10 Swiss female mice and one group of 9 female strain A mice) were exposed to high concentrations of chromium dust (7.8 to 13 mg Cr/m^3) in a nose-only chamber 0.5 hours/day, 5 days/week for 43, 52, and 20 weeks, respectively. No increase in the incidence of lung tumors was observed. It is unclear from the report whether the MTD was achieved by the dosing regimen used in these studies. Data on lung chromium content presented in the report indicated levels that were considerably lower than those observed in humans exposed occupationally. Ambiguity regarding the MTD and the variation in exposure periods used complicates the interpretation of the results.

Several studies assessed the carcinogenic potential of Cr(III) in the respiratory tract following exposure by intratracheal introduction, intrapleural injection, or intrabronchial implantation (Baetjer et al., 1959b; Hueper and Payne, 1962; Levy and Venitt, 1975; Levy and Martin, 1983). These studies did not report an increased incidence of tumors following exposure to trivalent chromium.

Baetjer et al. (1959b) suspended a chromium dust, similar in composition to that used in the inhalation studies, in olive oil, and zinc chromate and barium chromate in saline prior to intratracheal introduction into strain A, Swiss, and C57BL mice and mixed-breed rats (Wistar and McCollum stocks). The mice each received five to six installations of 0.01 to 0.05 mg chromium at 4- to 6-week intervals, while the rats received 15 introductions at the same dose at 2-week intervals. The total duration of the studies was between 32 and 52 weeks. The mice treated with chromium had a tumor incidence similar to age-matched controls, and the rats in both the treated and control groups had no benign or malignant tumors.

Hueper and Payne (1962) noted that no implantation site tumors were observed in 42 rats during a 24-month period following eight intrapleural implantations of 25 mg trivalent chromium acetate in gelatin over a 13-month period.

The National Institute for Occupational Safety and Health (NIOSH, 1975) criteria document on hexavalent chromium described a written communication (Levy and Venitt, 1975) reporting the results of a study performed at the Chester Beatty Research Institute in London. Random-bred Parton Wistar rats of both sexes received a pellet in the left inferior bronchiolus via tracheotomy under anesthesia. The rats were kept for 2 years. One hundred rats were used in the test group. The pellets that were implanted contained 2 mg ground chromite ore suspended 50/50 (weight/weight) in cholesterol. Negative control groups received either blank metal pellets or pellets and vehicle. Positive control groups received 3-methylcholanthrene. The lungs of all rats either dying during the study or killed at its termination were examined both macroscopically and microscopically. No bronchial carcinomas of the left lung were observed in the chromite ore test group.

Levy and Martin (1983) conducted an extensive investigation of 21 chromium-containing test materials in Wistar rats by intrabronchial implantation of a stainless steel wire mesh pellet containing 2 mg test material suspended in 2 mg cholesterol. The rats were allowed to live for 2 years, after which the study was terminated. No bronchial carcinomas were observed in the group receiving high silica chrome ore (III).

4.2.4. Subchronic Inhalation Studies

Data from subchronic animal studies identify the respiratory tract as the primary target of chromium toxicity following inhalation. Johansson et al. (1986) exposed rabbits to aerosols of hexavalent (0.9 mg/m^3 Na_2CrO_4) or trivalent (0.6 mg/m^3 $Cr(NO_3)_3$) chromium for 5 days/week, 6 hours/day for 4 to 6 weeks. The number of macrophages obtained from the lungs of the rabbits exposed to Cr(VI) was significantly increased. While the numbers of macrophages from rabbits exposed to Cr(III) were not increased, striking morphologic changes were observed, including round dark chromium-rich inclusions in the cytoplasm, an increased number of cells with a smooth inactive cell surface, enlarged Golgi apparatus, and a tendency toward elongated cell shape. The macrophages from rabbits exposed to Cr(VI) showed less marked morphologic changes than those exposed to Cr(III).

Johansson et al. (1980) exposed groups of four rabbits to chromium dust at concentrations of 3.1 mg/m^3 and 0.6 mg/m^3 for 5 days/week, 6 hours/day for 4 weeks. Macrophages collected from rabbits exposed to the higher concentration of chromium phagocytized significantly more chromium particles than the controls, although the number of nonviable macrophages was less than 3%.

Akatsuka and Fairhall (1934) exposed two cats to chromium carbonate dust at a level that varied from 3.3-83 mg/m^3 (average = 58.3 mg/m^3) for 86 sessions. Each session varied from 10-60 minutes, averaging 28 minutes for one cat and 57 minutes for the other. No effects in terms of gross or microscopic pathology were observed upon termination of the experiment. Examination of control animals, if there were any, was not reported.

Glaser et al. (1985) exposed 5-week-old male Wistar rats to aerosols of sodium dichromate at concentrations ranging from 0.025 to 0.2 mg Cr(VI)/m^3 22 hour/day in subacute (28 days) or subchronic (90 days) protocols. Subacute and subchronic exposures to Cr(VI) aerosol concentrations resulted in a positive correlation between exposure dose and significant effects on alveolar macrophages and immunologic function. Inhaled chromium was found to preferentially accumulate in the lung following exposure to chromate aerosols. Lung and spleen weights were significantly increased after both subacute and subchronic inhalation of chromate aerosols at concentrations greater than 0.025 mg/m^3. Serum contents of triglycerides and phospholipids differed significantly from controls ($p < 0.05$) in rats exposed subchronically to 0.2 mg/m^3 chromate. Inhalation of Cr(VI) aerosols stimulated the humoral immune system. Differences in the mean total serum immunoglobulin were significant at exposures above 0.025 mg/m^3, while exposures to aerosol concentrations greater than 0.1 mg/m^3 resulted in depression of the immune system stimulation. The primary antibody response to the B-cell-dependent antigen sheep red blood cell was elevated in a chromium-time and dose-dependent manner. The immune-stimulating effect of subchronic exposure to an aerosol with 0.05 mg/m^3 chromium was not reversed after 2 months of fresh air regeneration. The spleen T-lymphocyte subpopulation was also stimulated by subchronic exposure to 0.2 mg/m^3 chromium. Bronchoalveolar lavage (BAL) cell counts were significantly decreased following subchronic exposure to levels above 0.025 mg/m^3 chromium. The number of lymphocytes and granulocytes showed a slight but significant increase in the lavage fluids of the subacute and subchronically exposed groups. At subacute exposure concentrations up to 0.05 mg/m^3, the phagocytic activity of the alveolar macrophages increased; however, subchronic exposure at 0.2 mg/m^3 decreased this function significantly. Following subacute exposure to 0.2 mg/m^3 chromium, reductions in macrophage cell counts and phagocytic activities correlate with an observed lower clearance of inhaled iron oxide.

Glaser et al. (1990) exposed 8-week-old male Wistar rats to sodium dichromate at 0.05, 0.1, 0.2, and 0.4 mg Cr(VI)/m^3 22 hours/day, 7 days/week for 30-90 days. Chromium-induced effects were observed to occur in a strong dose-dependent manner. The authors observed obstructive respiratory dyspnea and reduced body weight following subacute exposure at the higher dose levels. The mean white blood cell count was increased at all doses ($p < 0.05$) and was related to significant dose-dependent leukocytosis following subacute exposures. Mean lung weights were significantly increased at exposure levels of 0.1 mg/m^3 following both the subacute

and subchronic exposures. Accumulation of macrophages was seen in all of the exposure groups and was postulated to be a chromium-specific irritation effect that accounted for the observed increases in lung weights.

Focal inflammation was observed in the upper airways following the subchronic exposure. BAL analyses provided more detailed information on the nature of the dichromate-induced irritation effect. BAL albumin was increased following the subacute exposure and was taken to indicate exudation into the alveolar region as an early irritation effect. The mean protein content of the cell-free lavage fluid was significantly increased in a dose-dependent fashion after the subacute and subchronic exposures. However, protein levels returned to control levels following a recovery period. Cytosolic lactate dehydrogenase and the number of mononuclear macrophages were also elevated following the subacute and subchronic exposures, particularly at the highest dose levels. The enzyme activity and number of macrophages returned to the control level following the recovery period. The authors concluded that chromium inhalation induced pneumocyte toxicity and suggested that inflammation is essential for the induction of most chromium inhalation effects and may influence the carcinogenicity of Cr(VI) compounds (Glaser et al., 1990).

Lee et al. (1988) exposed groups of 30 male and 30 female rats to 0.5 mg/m^3 or 25 mg/m^3 CrO_2 (IV) for 6 hours/day, 5 days/week for 2 years. Dust-laden alveolar macrophages with slight type II pneumocyte hyperplasia were noted following exposure at 0.5 mg/m^3. Inhaled particles were deposited mainly in the alveoli adjacent to the alveolar ducts, and the dust particles appeared as dense particles and were phagocytized by intra-alveolar macrophages. Exposure at 25 mg/m^3 overwhelmed the lung clearance mechanisms and resulted in significant increases in dust-laden macrophages, bronchioloalveolar cell hyperplasia with foamy macrophage response, and cholesterol granuloma in females in comparison with males. Two female rats developed well-differentiated cystic keratinizing squamous cell carcinomas with no tumor metastasis. The tumors were not characterized as neoplastic lesions.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Studies

No studies were located on reproductive or developmental effects in humans following exposure to Cr(III) compounds. Male and female rats treated with 1,806 mg Cr(III) kg/day as Cr(III) oxide 5 days/week for 60 days before gestation and throughout the gestation period had normal fertility, gestational length, and litter size (Ivankovic and Preussman, 1975).

Zahid et al. (1990) fed BALB/c albino Swiss mice trivalent (chromium sulfate) and hexavalent (potassium dichromate) chromium at concentrations of 100, 200, and 400 ppm for 35 days in the diet. The authors stated that the exposure groups included seven animals per group, and an additional seven animals were used as controls, although the report presents conflicting summaries of the actual group sizes throughout the report. Following the treatment, the authors examined the testes and epididymis of the animals. The epididymis was weighed and minced

suspended in buffered formalin. Sperm counts were then subsequently determined and sperm were examined for morphological abnormalities. Testes were fixed with Bouin's fluid for 1 week and were subsequently sectioned to 0.6 micron thickness and stained with haematoxylin and eosin for histologic examination. Ten sections were chosen randomly from the anterior, middle, and posterior parts of each testis and studied. One seminiferous tubule was chosen and examined to determine the cellular stages of spermatogenesis and the number of degenerated tubules. Statistical analyses of the data were conducted using the *t*-test between means and the 2 × 2 contingency Chi-square test between percentages. The authors reported deleterious effects on the male mouse testes, including ambiguous levels of degeneration in the outermost cellular layers of the seminiferous tubules, reduced (or absence of) spermatogonia per tubule, accumulation of germ cells in the resting spermatocytes stage, reduced sperm count in the epididymis, and increased percentage of morphologically abnormal sperm at all chromium sulfate dose levels. The authors concluded that the small but significant increase of hexavalent chromium in the testes of fed animals induced significant degeneration.

Serious questions have been raised regarding the design and conduct of this study (Finley et al., 1993; NTP, 1996a,b, 1997). The methods utilized by Zahid et al. (1990) are considered to be insufficient to identify spermatogonia, likely generated nonreproducible counts of epididymal sperm, and resulted in the biologically implausible conclusion of reduction in spermatogonia numbers concurrent with unchanged spermatocyte and spermatid numbers. Additional questions have been raised with regard to uncertainties regarding the actual groupings of animals used and the statistical analysis of the data (Finley et al., 1993).

Elbetieha and Al-Hamood (1997) examined fertility following chromium chloride exposures in mice. Sexually mature male and female mice were exposed to 1,000, 2,000, or 5,000 mg/L chromium chloride in drinking water for 12 weeks. The effects of the exposures on fertility were examined at 140 days. No mortality or clinical signs of toxicity were reported in any group of male or female mice exposed at any concentration in the experiment. The authors reported a number of effects, although in many cases the results were not strongly dose-dependent. The authors reported exposure of male mice to 5,000 ppm trivalent chromium compounds for 12 weeks had adverse impacts on male fertility. Testes weights were increased in the males exposed in the 2,000 and 5,000 mg/L dose groups, while seminal vesicle and preputial gland weights were reduced in the 5,000 mg/L exposed males. The number of implantation sites and viable fetuses were significantly reduced in females exposed to 2,000 and 5,000 mg/L chromium chloride. The authors did not report the amount of water ingested by the animals, other than to note that water ingestion was reduced in animals exposed to chromium. While the results of this study suggest the potential presence of reproductive effects, the lack of dose dependence in reported effects and the absence of data indicating actual exposures to the animals preclude the use of these data in risk assessment.

4.3.2. Inhalation Studies

No studies were located on reproductive or developmental effects in humans following exposure to Cr(III) compounds. No histopathologic abnormalities were observed in the testes of rats exposed to 0.1 mg total chromium (as a 3:2 mixture of Cr(VI) trioxide and Cr(III) oxide) for

18 months (Glaser et al., 1986, 1988). No additional data regarding the teratogenicity of inhaled trivalent chromium could not be located in the available literature.

4.4. OTHER STUDIES

4.4.1. Contact Dermatitis

Dermal exposure to chromium has been demonstrated to produce irritant and allergic contact dermatitis (Bruynzeel et al., 1988; Polak, 1983; Cronin, 1980; Hunter, 1974). Primary irritant dermatitis is related to the direct cytotoxic properties of chromium, while allergic contact dermatitis is an inflammatory response mediated by the immune system. Allergic contact dermatitis is a cell-mediated immune response that occurs in a two-step process. In the first step (induction), chromium is absorbed into the skin and triggers an immune response (sensitization). Sensitized individuals will illicit an allergic dermatitis response when exposed to chromium above a threshold level (Polak, 1983). Induction is generally considered to be irreversible. Chromium allergic dermatitis is characterized by symptoms of erythema, swelling, papules, small vesicles, dryness, scaling, and fissuring (Adams, 1990; MacKie, 1981).

Chromium is one of the most common contact sensitizers in males in industrialized countries (Fowler, 1990; Cronin, 1980) and is associated with occupational exposures to numerous materials and processes, including chrome plating baths, chrome colors and dyes, cement, tanning agents, wood preservatives, anticorrosive agents, welding fumes, lubricating oils and greases, cleaning materials, and textiles and furs (Burrows and Adams, 1990; Polak et al., 1973). Solubility and pH appear to be the primary determinants of the capacity of individual chromium compounds to elicit an allergic response (Fregert, 1981; Polak et al., 1973). The low solubility Cr(III) compounds are much less efficient contact allergens than Cr(VI) (Spruit and van Neer, 1966). While chromium compounds have been found to elicit an allergic response in occupational settings, this endpoint is not considered suitable for the development of a noncancer dose-response assessment.

4.4.2. Toxicant Interactions

Ascorbic, picolinic, and nicotinic acids have all been demonstrated to facilitate the absorption of Cr(III) through the intestinal wall (Anderson, 1997; ATSDR, 1993).

4.4.3. Genotoxicity

Trivalent chromium has been demonstrated to decrease the fidelity of DNA synthesis (Snow and Xu, 1991; Snow, 1994). Trivalent chromium chloride has been shown to produce genotoxic DNA adducts that inhibit DNA replication and are mutagenic (Snow, 1994). In general, trivalent chromium was not mutagenic in bacterial assays when tested with or without a mammalian activation system (Venitt and Levy, 1974; Petrilli and DeFlora, 1977, 1978a,b). In one study, trivalent chromium was mutagenic in *Baccillus subtilis*, but this activity was low compared with compounds of hexavalent chromium (Nakamuro et al., 1978).

There is conflicting information with regard to the ability of trivalent chromium to interact with DNA. Compounds of trivalent chromium were found to be clastogenic in BALB/c cells as $CrCl_3$ (Raffetto, 1977); CHO cells as $CrCl_3$, $Cr(NO_3)_3$, $KCr(SO_4)_2$, or $Cr(CH_3COO)_3$ (Levis and Majone, 1979); Don Chinese hamster cells as hydrated $CrCl_3$ (Ohno et al., 1982); and cultured human leukocytes as $Cr(CH_3COO)_3$ (Nakamuro et al., 1978). However, compounds of Cr(III) were not clastogenic in mouse FM3A cells as $Cr_2(SO_4)_3$ (Umeda and Nishimura, 1979), cultured human leukocytes as $CrCl_3$ or $Cr(NO_3)_3$ (Nakamuro et al., 1978), or Don Chinese hamster cells as $Cr_2(SO_4)_3$ (Ohno et al., 1982).

Cr(III) picolinate was shown to produce chromosome damage 3- to 18-fold above the control levels following soluble doses of 0.05, 0.1, 0.5, and 1.0 mM over a 24-hour treatment. The chromosome damage was inferred to result from the picolinate ligand following the demonstration of clastogenicity in the absence of Cr(III) (Stearns et al., 1995).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

4.5.1. Oral Studies

4.5.1.1. *Human Studies*

No human studies addressing noncarcinogenic effects of trivalent chromium were located in the available literature.

4.5.1.2. *Animal Studies*

Two oral chronic rodent bioassays of Cr(III) were located in the literature. Ivankovic and Preussman (1975) fed male and female rats up to 5% Cr_2O_3 baked in bread 5 days/week for 120 weeks. No adverse effects were noted at any feeding level. Schroeder et al. (1965) exposed mice and rats to drinking water containing 5 ppm Cr(III) for life. No increase in the incidence of tumors was seen in the treated animals with respect to controls.

Anderson et al. (1997) evaluated the subchronic toxicity of Sprague-Dawley rats fed 0-100 mg/kg Cr(III) chloride or Cr(III) tripicolinate in the diet for 24 weeks. No statistical differences in body weight or blood variables were noted among the groups examined at 11, 17, or 24 weeks. Histologic examination of the animals in the high-dose groups did not reveal any detectable differences. No toxicity was observed in any of the dose groups in this study.

4.5.2. Inhalation Studies

4.5.2.1. *Human Studies*

All of the available studies of occupational exposures include mixed exposures to both Cr(III) and Cr(VI). The Cr(VI) species has been suggested as the likely etiologic agent in reports of excess cancer risk in chromium workers; however, Cr(VI) cannot be unequivocally demonstrated to be the etiologic agent for noncarcinogenic effects following inhalation. While data addressing exposures to Cr(III) alone are not available, significant reduction of Cr(VI) to Cr(III) occurs in the lungs, and absorption of Cr(III) from lung tissue is known to occur (ATSDR, 1993; O'Flaherty, 1996). The following discussion presents results of studies involving mixed exposures to Cr(III) and Cr(VI).

4.5.2.1.1. ***Respiratory tract effects*****.** Three studies on chrome platers seem to provide some quantitative information on upper respiratory irritation after exposure to Cr(VI) as chromic acid. Cohen et al. (1974) reported that nasal ulcers and perforations were associated with total chromium concentrations of 1.4 to 43.9 $\mu g/m^3$, averaging 7.1 $\mu g/m^3$, and Cr(VI) concentrations of 0.09 to 9.1 $\mu g/m^3$, averaging 2.9 $\mu g/m^3$. The authors implicated direct contact, such as touching of the nose with chromium-contaminated hands, as a potentially important route of exposure. Lucas and Kramkowski (1975) reported similar results following worker exposure to Cr(VI) concentrations ranging from 1 to 20 $\mu g/m^3$, averaging 4 $\mu g/m^3$. Lindberg and Hedenstierna (1983) also found similar effects on nasal pathology and subjective symptoms. They reported reddening of the nasal mucosa at 1 to 2 $\mu g/m^3$, and nasal irritation (chronic and nasal septal ulceration and perforation) in two-thirds of the subjects at concentrations from 2 to 20 $\mu g/m^3$. Changes in vital capacity and forced expiratory volume were reported following Cr(VI) exposures greater than 2 $\mu g/m^3$.

4.5.2.1.2. ***Renal effects*****.** Cr(VI) exposure as low as 4 to 6 $\mu g/m^3$ has been reported to result in elevated excretion of β-2-microglobulin (Lindberg and Vesterberg, 1983b). The effect may be reversible since former chrome platers did not have an elevated concentration of either β-2-microglobulin or albumin in their urine.

In conclusion, effects on the airways and kidney have been observed in chrome platers exposed subchronically to chromic acid mist containing chromium in air at concentrations greater than 1 $\mu g/m^3$. Such effects include reddening of nasal mucosa, nasal irritation (ulceration, perforation), changes in pulmonary function, and renal proteinuria. Few of the available studies, however, provide quantitative concentration-response data on chromium health effects.

4.5.2.2. *Animal Studies*

In the only study specifically addressing noncarcinogenic effects by inhalation of Cr(III), Johansson et al. (1986) exposed rabbits to aerosols of trivalent chromium ($Cr(NO_3)_3$) at concentrations of 0.6 and 0.9 mg/m^3 for 6 hours/day, 5 days/week for 4-6 weeks. Striking morphologic changes were observed, including round dark chromium-rich inclusions in the

cytoplasm, an increased number of cells with a smooth inactive cell surface, enlarged Golgi apparatus, and a tendency toward elongated cell shape.

Johansson et al. (1980) exposed rabbits to chromium dust at 3.1 mg/m^3 and 0.6 mg/m^3 for 5 days/week, 6 hours/day for 4 weeks. Alveolar macrophages harvested from the exposed animals phagocytized significantly more chromium particles than those harvested from controls, although the number of nonviable macrophages was within the normal range (less than 3%) (Johansson et al., 1980).

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Applying the criteria for evaluating the overall weight of evidence for carcinogenicity to humans outlined in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), trivalent chromium is most appropriately designated a Group D–Not classified as to its human cartinogenicity. Using the *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), there are inadequate data to determine the potential carcinogenicity of trivalent chromium. The classification of hexavalent chromium as a known human carcinogen raises a concern for the carcinogenic potential of trivalent chromium. The inability to elucidate the contribution of trivalent chromium to cancer incidence following exposures to total chromium mixtures in these studies precludes a separate determination of whether trivalent chromium alone has carcinogenic potential. Data from oral and inhalation exposures of animals to trivalent chromium are inadequate to determine the carcinogenicity of trivalent chromium. The International Agency for Research on Cancer (IARC, 1990) concluded that animal data are inadequate for the evaluation of the carcinogenicity of Cr(III) compounds. Furthermore, although there is sufficient evidence of respiratory carcinogenicity associated with exposure to chromium, the relative contributions of Cr(III), Cr(VI), metallic chromium, or soluble versus insoluble chromium to carcinogenicity cannot be elucidated.

4.7. OTHER HAZARD IDENTIFICATION ISSUES

4.7.1. Possible Childhood Susceptibility

A number of factors may differentially affect the response of children to toxicants such as Cr(III). These factors include diet, physical environment, as well as maturation of physiological and biochemical processes. At present, there is too little information to make any statements about how these factors may specifically affect the toxicological responses of Cr(III) in children, be they cancer or noncancer.

4.7.2. Possible Sex Differences

At present, there is too little information to make any statements about the extent to which men differ from women in susceptibility to Cr(III) toxicity.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as carcinogenicity. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Oral RfD Background Document (on IRIS) for an elaboration of these concepts.

5.1.1. Choice of Principal Study and Critical Effect

Relatively few studies were located in the literature that addressed the oral toxicity of Cr(III). Ivankovic and Preussman (1975) fed rats baked bread containing up to 5% chromic oxide (Cr_2O_3) for 840 days. No effects due to Cr_2O_3 treatment were observed at any dose level. Ivankovic and Preussman (1975) also fed rats baked bread containing up to 5% Cr_2O_3 for 90 days. The only effects observed were reductions in the absolute weights of the livers and spleens of animals in the high-dose group. The absence of toxicity of trivalent chromium following these extraordinarily high doses likely reflect the low oral bioavailability of dietary Cr_2O_3.

In a subchronic study, Anderson et al. (1997) evaluated the toxicity of 0-100 mg/kg Cr(III) chloride and Cr(III) tripicolinate fed to Sprague-Dawley rats in the diet. Both of these forms of chromium have considerably higher bioavailability than that of Cr_2O_3. Histologic examination of the animals in the high-dose groups did not reveal any detectable differences with controls, and no toxicity was observed in any of the dose groups in this study. Dose levels in this study were considerably lower than those used by Ivankovic and Preussman (1975).

Elbetieha and Al-Hamood (1997) examined fertility following chromium chloride exposures in sexually mature mice at concentrations of 1,000, 2,000, or 5,000 mg/L chromium chloride in drinking water for 12 weeks. No mortality or clinical signs of toxicity were reported in any group of male or female mice exposed at any concentration in the experiment. The authors reported a number of effects on male fertility, although in many cases the results were not strongly dose dependent. The authors also reported a reduced number of implantation sites and viable fetuses in exposed females. The authors did not report the amount of water ingested by the animals, other than to note that water ingestion was reduced in animals exposed to chromium. While this study suggests the potential presence of reproductive effects, the lack of dose dependence in reported effects and the absence of data indicating actual exposures to the animals preclude the use of these data in risk assessment.

The Ivankovic and Preussman (1975) studies are considered to be most appropriate for development of the RfD.

5.1.2. Methods of Analysis

Ivankovic and Preussman (1975) fed groups of 60 male and 60 female rats chromic oxide (Cr_2O_3) baked in bread at dietary levels of 0%, 1%, 2%, or 5%, 5 days/week for 600 feedings (840 total days). Body weight and food consumption were monitored. The average total amounts of ingested Cr_2O_3 were given as 360, 720, and 1,800 g/kg bw for the 1%, 2%, and 5% treatment groups, respectively. The animals were maintained on control diets following termination of exposure until they became moribund or died. All major organs were examined histologically. Other toxicologic parameters were not mentioned explicitly but may have included some or all of those described for the accompanying subchronic study (see below). No effects due to Cr_2O_3 treatment were observed at any dose level.

Ivankovic and Preussman (1975) also treated rats (both sexes, 12-19 rats/group) at dietary levels of 0%, 2%, or 5% Cr_2O_3 in bread, 5 days/week for 90 days. Food consumption and body weight were monitored. Toxicologic parameters included serum protein, bilirubin, hematology, urinalysis, organ weights, and histopathology. With the exception of reductions (12%-37%) in the absolute weights of the livers and spleens of animals in the high-dose group, no effects could be detected that could be attributed to the Cr_2O_3 treatment.

5.1.3. RfD Derivation

No effects were reported at any dose level in Ivankovic and Preussman (1975) chronic study. The highest dose group (receiving 5% Cr_2O_3 in the diet for 600 feedings) can be considered a NOAEL for the study and was selected for derivation of the reference dose. The 5% dose group received an equivalent of 1,800 g/kg body weight. Adjustment of this dose level based on the amount of ingested Cr(III) (0.6849 Cr/g Cr_2O_3) and the feeding schedule (600 feeding days × 5 days/7 days) yields an adjusted NOAEL of 1,468 mg/kg-d. The lack of toxicity given the high concentrations of trivalent chromium used in the dose groups may reflect the poor absorption of trivalent chromium by the oral route of exposure (Visek et al., 1953; Mertz et al.,1965; MacKenzie et al., 1959; Ogawa, 1976; Henderson et al., 1979). Cr(III) has been found to be better absorbed in fasted than in fed rats (MacKenzie et al., 1959). The use of baked bread as a vehicle for dosing may have further reduced the absorption of chromium in the Ivankovic and Preussman (1975) study.

The adjusted NOAEL is further modified by two 10-fold uncertainty factors to account for the expected interspecies and interhuman variability in lieu of specific data. An additional 10-fold modifying factor is applied to reflect database deficiencies, including the lack of a study in a nonrodent mammal, lack of unequivocal data evaluating reproductive impacts, and the concern regarding potential reproductive effects raised by the study of Elbetieha and Al-Hamood (1997). The following additional uncertainties relate to the NOAEL derived from the Ivankovic and Preussman (1975) study: (1) the effects observed in the 90-day study were not explicitly addressed in the 2-year study; (2) the effect of the vehicle (baked bread) on absorption of chromium is uncertain, and the relevance of this dosing regimen to exposures in the environment is unclear; and (3) animals were allowed to die naturally after exposure stopped (2 years) and only then was histology performed. Application of the 100-fold uncertainty factor and 10-fold modifying factor to the adjusted NOAEL of 1,468 mg/kg-d gives the reference dose of 1.5 mg/kg-d.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

The inhalation RfC is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as carcinogenicity. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Studies of occupational exposure to chromium by inhalation all involve mixed exposures to both Cr(III) and Cr(VI). While data addressing exposures to Cr(III) alone are not available, significant reduction of Cr(VI) to Cr(III) occurs in the lungs, and absorption of Cr(III) from lung tissue is known to occur (O'Flaherty, 1996). Cr(VI) cannot be unequivocally demonstrated to be the sole etiologic agent for noncarcinogenic effects following inhalation.

Three studies on chrome platers reported upper respiratory irritation after exposure to Cr(VI) as chromic acid. In the study of Cohen et al. (1974), nasal ulcers and perforations were associated with total chromium concentrations of 1.4 to 43.9 $\mu g/m^3$, averaging 7.1 $\mu g/m^3$. Ninety-five percent of the 37 workers studied exhibited pathologic changes in nasal mucosa in a concentration-duration response. More than half of the workers employed less than 1 year had nasal pathology that was more severe than simple redness of the nasal mucosa. Almost all workers (35 of 37) employed longer than 1 year had nasal tissue damage. The authors implicated direct contact, such as touching of the nose with chromium-contaminated hands, as a potentially important route of exposure. Lucas and Kramkowski (1975) revealed similar results. Lindberg and Hedenstierna (1983) also found similar effects on nasal pathology and subjective symptoms. All workers with nasal ulceration had been exposed to chrome acid mist, which contained Cr(VI) at 20 $\mu g/m^3$, or greater near the baths. Changes in vital capacity and forced expiratory volume were seen following Cr(VI) exposures greater than 2 $\mu g/m^3$.

Only one animal study addressed exposure to Cr(III) by inhalation. Johansson et al. (1986) reported the results of a subchronic study in which rabbits were exposed to aerosols of trivalent chromium ($Cr(NO_3)_3$) at concentrations of 0.6 and 0.9 mg/m^3 for 6 hours/day, 5 days/week for 4-6 weeks. Striking morphologic changes were observed, including round dark chromium-rich inclusions in the cytoplasm, an increased number of cells with a smooth inactive cell surface, enlarged Golgi apparatus, and a tendency toward elongated cell shape. This study utilized small groups and focused on endpoints that are not considered to be appropriate for development of an RfC for Cr(III).

Data are considered to be inadequate for development of an RfC due to the lack of a relevant toxicity study addressing respiratory effects of Cr(III). The currently available data are inadequate to elucidate the contribution of trivalent chromium to respiratory effects observed following occupational exposures to mixtures of trivalent and hexavalent chromium. The one animal study that evaluated the toxicity of trivalent chromium by the inhalation route of exposure (Johansson et al., 1986) utilized only one exposure concentration and did not identify a lowest-observed-adverse-effect level. As a result, this study is considered inadequate to support the

development of an RfC for trivalent chromium, and an RfC for trivalent chromium cannot be developed at this time.

5.3. CANCER ASSESSMENT

Occupational exposure to airborne chromium has been studied in the chromate manufacturing and ferrochromium industries; in all cases exposures all include mixed exposures to both Cr(III) and Cr(VI). Data addressing exposures to Cr(III) alone are not available. Cr(VI) has been classified as a known human carcinogen, and the contribution of Cr(III) to the observed lung cancer in these populations cannot be elucidated from these studies. Animal data are inadequate for the evaluation of the carcinogenicity of Cr(III) compounds. The two oral studies located in the available literature (Schroeder et al., 1965; Ivankovic and Preussman, 1975) reported negative results for rats and mice. Several animal studies have been performed to assess the carcinogenic potential of Cr(III) by inhalation. These studies have not found an increased incidence of lung tumors following exposure either by natural routes, intrapleural injection or intrabronchial implantation (Baetjer et al., 1959b; Hueper and Payne, 1962; Levy and Venitt, 1975; Levy and Martin, 1983). For the reasons stated above, a quantitative dose-response assessment has not been generated for Cr(III).

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

6.1. HUMAN HAZARD POTENTIAL

Chromium is a naturally occurring element that may exist in several chemical forms and valence states in the environment. The most commonly occurring valence states are chromium metal (0), trivalent chromium (III), and hexavalent chromium (VI). Cr(III) potentiates the action of insulin in peripheral tissue and is essential for animals and humans. Adults in the United States are estimated to ingest approximately 60 µg/day of chromium from food (ATSDR, 1993).

NRC has identified an ESADDI for chromium of 50-200 µg/d (NRC, 1989), corresponding to 0.71-2.9 µg/kg/day for a 70 kg adult. FDA has selected a Reference Daily Intake for chromium of 120 µg/d (U.S. DHHS, 1995).

The bioavailability of chromium may be the single most important factor determining the toxicity of a specific chromium source (O'Flaherty, 1996). Ingested hexavalent chromium is efficiently reduced to the trivalent form in the gastrointestinal tract. Gastrointestinal absorption of Cr(VI) occurs with greater efficiency than absorption of Cr(III), and absorption of ingested trivalent chromium is estimated to be less than 3%. Trivalent chromium is absorbed very slowly by inhalation. Following inhalation exposure, chromium may be absorbed into the systemic circulation, transferred to the gastrointestinal tract by mucociliary action, or remain in the lung.

A significant amount of absorbed chromium is taken up in the bone, liver, kidney, and spleen. Hexavalent chromium readily crosses cell membranes through the phosphate and sulfate anion-exchange carrier pathway. Cr(III) compounds may cross cell membranes, but only with very low efficiency. There is conflicting information regarding the ability of trivalent chromium to interact with DNA. In general, trivalent chromium was not mutagenic in bacterial assays when tested with or without a mammalian activation system.

Occupational exposure to trivalent chromium and other chromium compounds by inhalation has been studied in the chromate manufacturing and ferrochromium industries; however, all studies include mixed exposures to both Cr(III) and Cr(VI). While the Cr(VI) species is the likely etiologic agent in reports of excess cancer risk in chromium workers, studies are inadequate to rule out a contribution of Cr(III) to chromium carcinogenicity, and data addressing exposures to Cr(III) alone are not available.

Relatively few studies were located in the literature that addressed the oral or inhalation toxicity of Cr(III). No effects other than reductions of the absolute weights of livers and spleens of rats have been observed following oral exposure to Cr(III). While striking morphologic changes occurred in the macrophages of rabbits exposed to Cr(III) aerosols in the one inhalation study of Cr(III) in animals, the database is inadequate to support development of an RfC for Cr(III).

Chromium is one of the most common contact sensitizers in industrialized countries, and allergic contact dermatitis is associated with occupational exposures to numerous materials and processes, including chrome plating baths, chrome colors and dyes, cement, leather tanning agents, and wood preservatives.

6.2. DOSE RESPONSE

Studies of inhalation of mixtures of trivalent and hexavalent chromium in occupational populations support the classification of Cr(VI) as a known human carcinogen but provide inadequate data for evaluation of carcinogenicity of Cr(III) compounds.

The database on noncarcinogenic effects of Cr(III) is also lacking. Relatively few studies were located in the literature that addressed the oral toxicity of Cr(III). Of these, the Ivankovic and Preussman (1975) study was judged to be the only study suitable for development of an RfD for Cr(III). The confidence in the RfD developed using the Ivankovic and Preussman (1975) study is low. The primary purpose of the Ivankovic and Preussman (1975) study was to assess the carcinogenic potential of Cr_2O_3. The confidence in the principal study is considered low because of the lack of explicit detail on study protocol and results. No effects due to Cr_2O_3 treatment were observed at any dose level in this study. No macroscopic or histologic signs of toxicity were noted in the 2-year study, although the effects observed in the 90-day study were not explicitly addressed. Animals in this study were allowed to die naturally after exposure stopped (2 years) and only then was histology performed. Data on potential reproductive and developmental effects of Cr(III) are lacking. A modifying factor of 10 was applied to the

adjusted NOAEL to account for the potential reproductive toxicity identified by the study of Elbetieha and Al-Hamood (1997) and the absence of a credible study addressing reproductive endpoints. The adjusted NOAEL is further modified by two 10-fold uncertainty factors to account for the expected interspecies and interhuman variability in lieu of specific data. Only one study was located that specifically addressed noncarcinogenic effects of Cr(III) by the inhalation route of exposure (Johansson, 1986). While this study reported striking morphologic changes in the macrophages of rabbits exposed to Cr(III) aerosols, the database is inadequate to support development of an RfC for Cr(III). Nasal septum irritation, atrophy, and perforations have been widely reported following mixed exposures to hexavalent and trivalent chromium in the occupational setting. The available studies are inadequate to determine whether trivalent chromium contributed to the observed effects.

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

The support document and IRIS summary for trivalent chromium have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review. Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

Comments on General Questions

1. *Are you aware of any other data/studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?*

A. Comment: A new report has been found showing significant embryotoxic and fetotoxic damage due to exposure of rats and mice to high doses of Cr(VI) or Cr(III) in drinking water. I don't feel confident rederiving an RfD based on these data, and the doses are clearly very high. However, derivation of an RfD based on an observed toxicological effect appears to be preferable to an RfD based on a NOAEL.

Response to Comment: The report has been added to the reproductive/developmental studies section of the toxicological review document. The study reports on a variety of embryotoxic and fetotoxic endpoints; however, many of the observed effects did not occur in a clear dose-dependent fashion. Further, the authors did not indicate the amount of water ingested by the animals and only stated that water ingestion was reduced in the treatment groups relative to the controls. This omission precludes determination of the actual doses experienced by the treated animals. This study is not considered to be sufficient for development of the RfD for Cr(III).

B. Comment: The study by Baetjer et al. (1959b) needs some more discussion regarding the question as to whether the MTD has been achieved in this particular study. From the published paper there is no indication that, indeed, the animals had been sufficiently dosed, and it is therefore questionable whether this study should be labeled an adequate carcinogenicity study.

Response to Comment: The discussion has been added to the text.

C. Comment: The documents correctly state that Cr(VI) gets transformed to Cr(III) in vivo, but they skirt the issue of whether or not a Cr(VI) study is really a study of in vivo exposure to Cr(III).

Response to Comment: Given the rapid reduction of Cr(VI) to Cr(III) in vitro, it is relevant to consider whether environmental exposures to Cr(VI) or administration of Cr(VI) in controlled animal experiments is essentially identical to environmental exposures to Cr(III) or administration of Cr(III) in controlled experiments. While considerably more data are available for Cr(VI) than for Cr(III), it appears at present that exposures to Cr(VI) have considerably different outcomes than exposures to Cr(III). The Agency has prepared the Toxicological Reviews and IRIS files for Cr(VI) and Cr(III) from this perspective.

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

A. Comment: I believe the Agency is a bit conservative regarding the confidence of the RfD. The Ivankovic and Preussman (1975) study appears to be done according to acceptable practices at the time. I also take issue with the modifying factors. The Agency used a modifying factor of 10 to reflect uncertainty in the database. I disagree with the speculation that the NOAEL could be a LOAEL. The fact that uptake is low and variable does not justify such a high uncertainty factor, and the fact that the animals died naturally if anything would tend to increase disease incidence rates. I believe a threefold modifying factor is sufficient. I would also note that the authors arbitrarily dropped the RfD by an additional 1/3 by rounding down from 1.468 to 1. A more realistic estimate might be 1,468/300 = 5 mg/kg-day.

Response to Comment: In order to be conservative regarding the limited database on Cr(III), the Agency supports the use of a 10-fold modifying factor to reflect uncertainty in the database, including uncertainty resulting from the deficiencies in the database addressing potential reproductive and developmental effects. The speculation that the NOAEL could be a LOAEL has been removed from the document. The RfD has been rounded to 1.5.

B. Comment: In consideration of uncertainties surrounding the critical study for the RfD, such as the appropriateness of the method of administration (in baked bread, which might be expected to have a lesser bioavailability than if administered in feed or water), the use/application of a modifying factor of 3 (reducing the RfD to about 0.3 mg/kg-day) seems appropriate.

Response to Comment: The derivation of the RfD using the data of Ivankovic and Preussman (1975) incorporated a 10-fold uncertainty factor to account for uncertainties in the study, including the likely low absorption of chromium from the baked bread. The Agency considers the current uncertainty factors to be sufficient for development of the RfD.

3. *Have the noncancer and cancer assessments been based on the most appropriate studies? These studies should present the critical effect/cancer (tumors or appropriate precursor) in*

the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

A. Comment: The RfC should be for total chromium since it is not clear that soluble chromium species make up all of the hexavalent chromium species in the mixture, nor that insoluble chromium species consist solely of trivalent species. Furthermore, the data suggest that there might be a positive interaction between the two forms of chromium and that the risk for exposure to a mixture is not the sum (or average) of the risks from either species. However, the available data do not provide any evidence that trivalent chromium alone is toxic by inhalation. The data are inconclusive with regard to inhalation exposure to trivalent chromium.

Response to Comment: Considerable data are available regarding the effects of inhalation of hexavalent chromium in the form of chromic acid mists and hexavalent chromium particulates. The limited data available for trivalent chromium suggest that this compound may behave essentially as a nuisance dust upon inhalation. The Agency considers the data to be insufficient to support the application of the RfCs derived for hexavalent chromium to the trivalent species.

4. *Studies included in the RfD and RfC under the heading "Supporting/Additional Studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, any evidence corroborative of the critical effect, or to establish the comprehensiveness of the database with respect to various endpoints (such as reproductive/developmental toxicity studies). Should other studies be included under the "Supporting/Additional" category? Should some studies be removed?*

A. Comment: Some of the statements related to the genotoxic effects of trivalent chromium are either inaccurate or misleading.

Response to Comment: The recommended modifications to this section have been made.

B. Comment: The new data on reproductive toxicity of chromium in the drinking water needs to be carefully compared to the NTP study in which rats and mice were fed potassium chromate in the diet. The form of chromium and route of exposure are clearly of paramount importance.

Response to Comment: A discussion comparing and contrasting the results of the NTP studies and the new report has been added to the reproductive/developmental studies section of the Toxicological Review document. The report of Elbetieha and Al-Hamood (1997) raises the possibility of reproductive effects at very high doses, but the observed endpoints do not show a clear dose response, and the authors did not provide the data on drinking water ingestion required to determine the doses experienced by the treatment groups. This study is not considered sufficient for development of the RfD.

5. *For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors or the modifying factor? Do you consider that the data support the use of different (default) values than those proposed?*

A. Comment: The appropriate data have been considered in developing the uncertainty factors and the modifying factor.

6. *Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the database? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?*

A. Comment: The evidence for the role or lack of role of trivalent chromium in human cancer is not convincingly presented. In particular the lack of effect of feeding rats chromium-laden bread is not balanced by any demonstration of chromium uptake in the animals.

Response to Comment: The Toxicological Review document concludes that there are inadequate data to determine the potential carcinogenicity of trivalent chromium. This should not be construed as a conclusion that trivalent chromium is not carcinogenic.

Comments on Chemical-Specific Questions

1. *Are the conclusions of Zahid et al. (1990) regarding potential reproductive toxicity of Cr(III) in any way countered by the results of the NTP study?*

A. Comment: If NTP was not able to reproduce Zahid's results, I do not believe Zahid's results should be considered.

B. Comment: The Zahid et al. studies are supported, but in a more realistic fashion by the studies of Juniad and Kanojia.

Response to Comments: The studies of Zahid et al. (1990) and Juniad et al. and Kanojia et al. (1996) did not involve common endpoints. The NTP study was unable to repeat the findings of Zahid et al. The results of Juniad and Kanojia provide evidence of reproductive and fetotoxic effects of Cr(VI) at high doses but do not support the findings of Zahid et al. regarding toxicity of Cr(III).

C. Comment: Additional description should be provided regarding the report of Zahid et al. in relation to the NTP study.

Response to Comment: Additional description has been added.

2. Are there any studies available which could be used to develop an RfC for trivalent chromium?

A. Comment: I agree with the document that there are no adequate data for Cr(III).

B. Comment: I agree with EPA's assessment that there are no suitable data sets to use in calculating an RfC for airborne Cr(III).

Response to Comments: An RfC for airborne Cr(III) has not been developed.

3. The principal study (Mancuso, 1975) and the follow-up study (Mancuso, 1997) show the best dose-response relationship for total chromium, but animal data only support a conclusion of carcinogenicity of hexavalent chromium. Should the potency estimate address total chromium or hexavalent chromium?

A. Comment: Mancuso alleges that his data support a conclusion that both Cr(III) and Cr(VI) are carcinogens. His data set is very small, and in my view, it lacks the power to distinguish between Cr(VI) and total chromium. The animal data present a convincing story that Cr(III) is not carcinogenic. Hence, I believe the potency estimates should address only hexavalent chromium. EPA should be more explicit as to why they discount Mancuso's allegation.

Response to Comment: Additional commentary has been added to the section addressing the conclusion that only Cr(VI) is known to be carcinogenic by the inhalation route of exposure.

B. Comment: The potency estimate should be based on total chromium but should note that the exposure is mixed (and give the relative proportions of Cr(III) and Cr(VI) to which the workers were exposed). Although it is probably true that the carcinogenicity is due to the hexavalent chromium, the finding that trivalent chromium can be taken up by the aveolar macrophages could imply that the carcinogenic process may be modulated by the presence of trivalent chromium.

Response to Comment: The Agency acknowledges that the cohort was occupationally exposed to mixtures of trivalent and hexavalent chromium, and agrees that it is probably true that the carcinogenicity is due to the hexavalent chromium. The ratio of trivalent and hexavalent chromium in the mixture have been provided. However, the Agency considers the data on carcinogenicity of trivalent chromium to be insufficient to support a conclusion that trivalent chromium contributed to the cancer observed in the cohort. The Agency concluded that Cr(VI) is known to be carcinogenic in humans and that data are insufficient to form a conclusion regarding the potential carcinogenicity of trivalent chromium.

C. Comment: It is obvious that based on the human data hexavalent chromium is a Group A carcinogen. However, because of the insufficient data, a contribution of the trivalent chromium cannot be ruled out since the workers are always exposed to a mixture of both chromium types.

Response to Comment: The Toxicological Review document concludes that there are insufficient data to determine whether trivalent chromium is a carcinogen. This conclusion should not be misconstrued as ruling out a role for trivalent chromium in carcinogenicity.

4. There is a Canadian study that relates stomach cancer to gold mining following exposures to chromium. Does this study justify/support determination of an oral factor for chromium?

A. Comment: No.

Response to Comment: The Canadian study has not been used to develop an oral slope factor for chromium.

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