EPA/635/R-10/004A
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TOXICOLOGICAL REVIEW

OF

HEXAVALENT CHROMIUM

(CAS No. 18540-29-9)

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

September 2010

(Note: This document is a reassessment of the noncancer and cancer health effects associated with the oral route of exposure only.)

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U.S. Environmental Protection Agency
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LIST OF ABBREVIATIONS AND ACRONYMS

3β-Δ5-HSH	3β-Δ^5-hydroxysteroid dehydrogenase
AAS	atomic absorption spectrometry
AcP	acid phosphatase
ADAF	age-dependent adjustment factor
AIC	Akaike's Information Criterion
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BER	base excision repair
BMD	benchmark dose
BMDL	95% lower confidence limit of benchmark dose
BMDS	benchmark dose software
BMR	benchmark response
CalEPA	California Environmental Protection Agency
CASRN	Chemical Abstracts Service Registry Number
CCA	chromated copper arsenate
CI	confidence interval
Cr	chromium
CSF	cancer slope factor
DNA	deoxyribonucleic acid
ED	Enumeration Districts
EDTA	ethylenediaminetetraacetic acid
EPR	electron paramagnetic resonance
ER	excision repair
FSH	follicle-stimulating hormone
GD	gestation day
GFR	glomerular filtration rate
GH	growth hormone
GI	gastrointestinal
GSH	glutathione
GST	glutathione S-transferase
H&E	haematoxylin and eosin
HCl	hydrochloric acid
Hct	hematocrit
Hgb	hemoglobin
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
ICP-AES	inductively coupled plasma atomic emission spectrophotometry
ICP-OES	inductively coupled plasma optical emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
i.m.	intramuscular
i.p.	intraperitoneal
i.t.	intratracheal
i.v.	intravenous
IRIS	Integrated Risk Information System

K_m	Michaelis constant
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean cell volume
MMAD	mass mean aerodynamic diameter
MMR	mismatch repair
NER	nucleotide excision repair
NJDEP	New Jersey Department of Environmental Protection
NJDOH	New Jersey Department of Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
OPP	Office of Pesticide Programs
PAM	pulmonary alveolar macrophages
PBPK	physiologically based pharmacokinetic
PEG	polyethylene glycol
PND	postnatal day
POD	point of departure
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SDH	sorbitol dehydrogenase
SMART	somatic mutation and recombination test
TEM	transmission electron microscopy
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
V_{max}	maximum velocity of enzyme reaction
WBC	white blood cell

FOREWORD

The purpose of this Toxicological Review is to provide the scientific support and rationale for the hazard identification and dose-response assessments in IRIS pertaining to chronic exposure to hexavalent chromium via ingestion. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of hexavalent chromium. This document is a reassessment of the noncancer and cancer health effects associated with the oral route of exposure. A reassessment of the noncancer and cancer health effects of hexavalent chromium associated with the inhalation route of exposure will be conducted at a later date.

Section 5, *Dose-Response Assessments*, is based largely on the work of four independent groups that have recently evaluated the toxicity of hexavalent chromium via ingestion: (1) U.S. EPA's Office of Pesticide Programs (OPP), (2) the New Jersey Department of Environmental Protection (NJDEP), (3) the California Environmental Protection Agency (CalEPA), and (4) the Agency for Toxic Substances and Disease Registry (ATSDR). Section 5.1 relies, in part, on work conducted by ATSDR and CalEPA, and the reference dose (RfD) generally relies on ATSDR's analysis for chronic oral exposure to hexavalent chromium. Section 5.3 was developed, in part, based on work conducted by CalEPA and NJDEP, and the oral cancer slope factor (CSF) generally relies on NJDEP's analysis for cancer potency.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (e-mail address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard identification and dose-response assessments of ingested hexavalent chromium. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment. This *Toxicological Review of Hexavalent Chromium* provides documentation for oral toxicity values only (i.e., RfD and oral cancer slope factor). A reassessment of the noncancer and cancer health effects of hexavalent chromium associated with the inhalation route of exposure will be conducted at a later date.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as 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 (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. 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). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless indicated otherwise, the RfD and RfC are derived for chronic exposure durations.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. 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 may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for hexavalent chromium has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk*

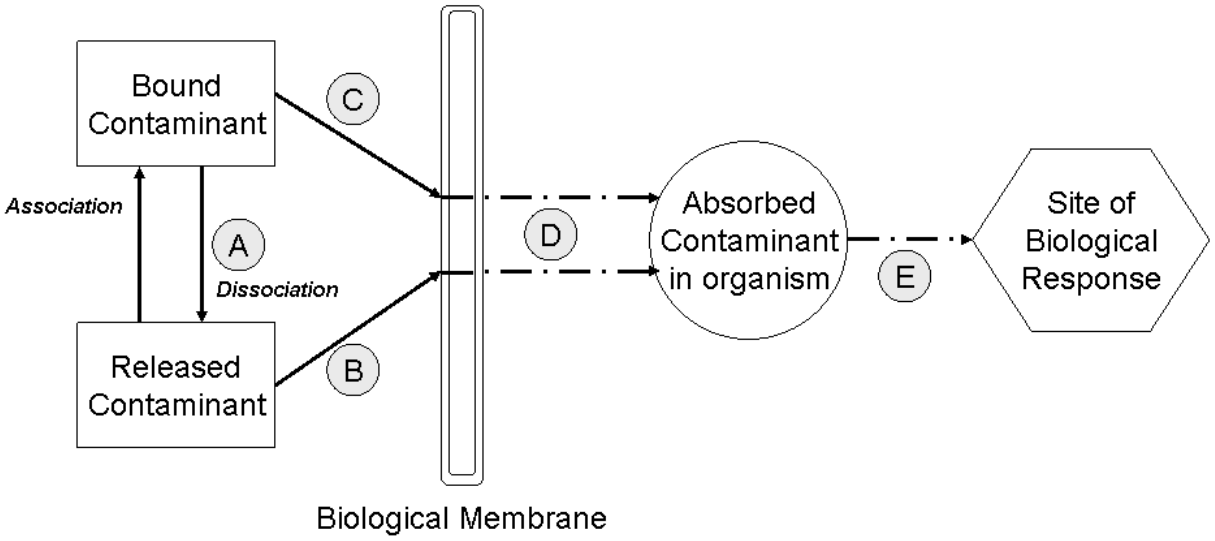
Assessment of Chemical Mixtures (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim 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), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through September 2010.

2. CHEMICAL AND PHYSICAL INFORMATION

This toxicological review restricts its focus to oral exposure to hexavalent chromium compounds. Hexavalent chromium compounds are a group of substances that contain chromium in the hexavalent or +6 oxidation state. Hexavalent chromium compounds discussed in this document include the following: chromium(VI) oxide, chromic acid, and selected salts of the chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) anions.

This section discusses sources of chromium in the environment and the physicochemical properties of chromium compounds that determine their environmental bioaccessibility. It is recognized that the definition of bioaccessibility and bioavailability has differed across disciplines and can cause confusion especially in a regulatory context (Semple et al., 2004). For the purposes of this toxicological review, bioaccessibility is defined as the ability of chromium to be released (e.g., environmental solubilization and reduction or extracellular digestion and reduction) from the environmental matrix to which it is bound, i.e., the fraction of the dose ingested that becomes freely available for absorption via crossing a cellular membrane. As shown in Figure 2-1, this would encompass processes A through C. Key determinants of environmental bioaccessibility include the following: 1) type of contaminant, 2) contamination level, 3) type of soil, 4) pH of soil, 5) aging of soil, and 6) metal speciation. Processes that control the environmental chemistry of chromium include redox transformation, precipitation/dissolution, and adsorption/desorption reactions.



Adapted from: Semple et al. (2004) and NRC (2002).

Figure 2-1. Definitions of bioaccessibility and biovailability used in this document.

Processes that determine exposure to a contaminant include release of soil-bound contaminant (A), and subsequent transport (B), or transport of bound contaminant (C), uptake across a physiological membrane (D), and incorporation into a living system (E). For the purposes of this toxicological review, environmental and extracellular processes A through C are defined as bioaccessibility. Environmental bioaccessibility is discussed in this section. Extracellular bioaccessibility processes that also may occur within an organism, for example in the GI lumen, are discussed in Section 3 on toxicokinetics. Processes D and E, represented by the dashed lines, are defined as bioavailability and are also discussed in Section 3.

Processes A through C can also occur internal to an organism, and the same determinants of bioaccessibility function in that internal environment, e.g., pH in different sections of the gastrointestinal (GI) lumen. Both environmental and internal bioaccessibility processes are extracellular. For the purposes of this Toxicological Review, however, this latter internal, extracellular bioaccessibility is discussed in Section 3 on toxicokinetics.

Bioavailability (processes D and E in Figure 2-1) is defined in this document as the potential for chromium to cross cellular boundaries, i.e., the degree to which it becomes available to the target tissue after administration. Key mechanisms of bioavailability that determine internal tissue dose include the following: uptake through cell membranes, intracellular

distribution, and binding to cellular macromolecules. These bioavailability processes are also discussed in Section 3. The toxicodynamics of responses to that internal tissue dose are discussed in Section 4.

2.1. ENVIRONMENTAL SOURCES AND OCCURRENCE

Elemental chromium was first discovered and characterized by the French chemist Nicolas-Louis Vauquelin in Siberian red lead ore (crocoite) in 1797 (Katz and Salem, 1993; Costa and Klein, 2008). It is a naturally occurring element present in the earth's crust. Chromium ranks 21st among elements in crustal abundance (Krausopf, 1979), and is found in virtually all phases including air, water, soil and biota (Losi et al., 1994). The average chromium concentration in the continental crust has been commonly observed to range from 80 to 200 mg/kg (NAS, 1974).

The chromium content of soil is largely dependent on the parent materials, with an average reported as 40 mg/kg (Bertine and Goldberg, 1971). The average concentration of chromium in freshwater is 1.0 µg/L (range: 0.1–6.0 µg/L), while the average concentration of chromium in seawater is 0.3 µg/L (range: 0.2–50 ug/L) (Bowen, 1979 as cited in Losi et al., 1994). Drainage water from contaminated areas may have higher concentrations. The aqueous concentrations of chromium and its mobility in different geologic environments are dependent on its oxidation state (Rai et al., 1989).

Hexavalent chromium compounds are well-known as laboratory reagents and as manufacturing intermediates. Selected industrial uses of hexavalent chromium compounds are provided in Table 2-1. The major industries using chromium are the metallurgical, chemical, and refractory brick industries (Langard, 1980 as cited in Losi et al., 1994). Major uses of hexavalent chromium compounds include metal plating, manufacture of pigments and dyes, corrosion inhibitors, chemical synthesis, refractory production, leather tanning, and wood preservation (Blade et al., 2007; Shanker et al., 2005; Page and Loar, 2004). Sodium chromate, sodium dichromate, and chromium(VI) oxide are obtained directly from chromite ore through an oxidative alkaline roasting process (Anger et al., 2005; Page and Loar, 2004). Sodium chromate and sodium dichromate are the starting materials for the production of most other chromium compounds (Anger et al., 2005; Page and Loar, 2004). Hexavalent chromium compounds are classified as oxidizing agents (Anger et al., 2005; Cotton et al., 1999). Chromium(VI) oxide and ammonium dichromate can react explosively when brought into contact with organic materials (Lewis, 2007; O'Neil, 2006).

Table 2-1. Industrial uses of hexavalent chromium compounds

Name	Formula	Uses
Chromium oxide	Cr_2O_3	Various metallurgical uses including production of ferroalloys used to make stainless steel and nonferrous alloys or superalloys, such as those used in jet engines
Barium oxide	$BaCrO_4$	Pyrotechnics, high-temperature batteries
Cadmium chromate	$CdCrO_4$	Catalysts, pigments
Cadmium dichromate	$CdCr_2O_7:H_2O$	Metal finishing
Calcium chromate	$CaCrO_4$	Metal primers, corrosion inhibitors, high-temperature batteries
Copper dichromate	$CuCr_2O_7:2H_2O$	Wood preservatives, catalysts
Magnesium chromate	$MgCrO_4:5H_2O$	Corrosion inhibitor in photoengraving, ceramics
Mercuric chromate	$HgCrO_4$	Antifouling formulation
Pyridine dichromate	$(C_5H_5NH)_2:Cr_2O_7$	Photosensitizer in photoengraving, cermics
Strontium chromate	$SrCrO_4$	Corrosion-inhibiting pigment, plating additive

Source: Hartford (YEAR) as cited in Nriagu (1988).

Alloys with iron, nickel or cobalt are prepared from metallurgical grade ore (60% ≥ chromic oxide) for use in the production of a wide variety of steels including stainless steel, austenite steel, and high-speed and high-temperature steels, and in other nonferrous alloys. The chemical industry generally uses a lower grade chromite ore (≈ 45% chromic oxide) to synthesize sodium chromate and dichromate from which most other chromium products are prepared, including products used in pigment manufacture, plating/metal finishing, corrosion inhibition, organic synthesis, leather tanning, and wood preservation. Other important anthropogenic sources of chromium in the environment include fuel combustion, cement production, and sewage sludge incineration/deposition (U.S. EPA, 1984).

Chromated copper arsenate (CCA) wood preservatives to prevent fungal decay and infestations by wood-boring insects contain hexavalent chromium. The preservatives are used in the industrial vacuum-impregnation of timber and supplied as pastes or water-based concentrates that are diluted to between 1 and 10% w/w total salts (Cocker et al., 2006). Wood preservatives such as CCA are regulated under the Control of Pesticide Regulations 1986, with only approved compounds placed on the market. Cocker et al. (2006) showed that workers exposed to CCA wood preservatives have urinary chromium levels significantly higher than nonoccupationally exposed populations, but below occupational biological exposure indices for urinary chromium based on inhalation exposures. Balasoiu et al. (2000) evaluated the influence of soil composition and physicochemical characteristics on the retention and partitioning of chromium in nine CCA artificially contaminated soils using a statistical mixture design to arrive at the different soil compositions. Sequential extraction and modified solvent extraction were used to assess the partitioning. Average metal retention in mineral soils was low (23%), but increased dramatically

in highly organic soils (78%). Levels of chromium in soluble or exchangeable form were very low in highly organic soils. Conversely, 18% of chromium in mineral soils was found in exchangeable form. Thus, chromium in moderately and highly organic contaminated soils was present in less mobile and less bioavailable forms; in mineral soils the labile fraction was higher.

Food is also a major source of exposure to chromium. Daily oral intake rates for populations in the United Kingdom (U.K.) were estimated by Rowbotham et al. (2000) for infants (1 year), children (11 years) and adults to be 33–45, 123–171, and 246–343 μg/person/day, respectively. Plessi and Monzani (1990) showed that chromium content in whole cereals differed substantially and was mostly concentrated in pericarps. Variations occurred not only among different types of cereals, but also within cereals of the same type depending on the area of origin. Kumulainen (1991) showed that food processing may increase food chromium content, depending on the process. Processes such as meat grinding and homogenization using stainless-steel equipment increased the chromium content of foods. Acidic fruit juices in contact with steel cans are high in chromium as well.

2.2. ENVIRONMENTAL CHEMISTRY, SPECIATION, AND BIOACCESSIBILITY

Chromium is a first series transition element for group VIA on the periodic table. In its elemental form, it is a hard, white, lustrous, and brittle metal with a high melting point (2000°C) (Costa and Klein, 2006). Chemical structures and selected physical and chemical properties of selected hexavalent chromium compounds are presented in Table 2-2, and the standard potential for the oxidation-reduction equilibria among the valence states are summarized in Table 2-3.

Table 2-2. Physical properties of selected hexavalent chromium compounds

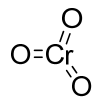
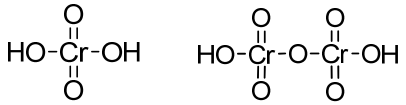
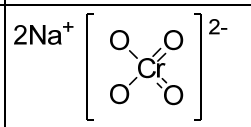
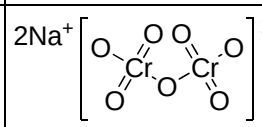
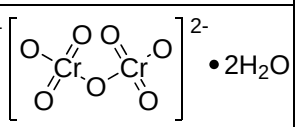
Name	Chromium(VI) oxide[a]	Chromic acid[a,b]	Sodium chromate	Sodium dichromate	Sodium dichromate, dihydrate
CASRN	1333-82-0	7738-94-5 (H_2CrO_4); 13530-68-2 ($H_2Cr_2O_7$)	7775-11-3	10588-01-9	7789-12-0
Synonyms (ChemID Plus, 2008)	Chromium oxide; hexavalent chromium oxide; chromic trioxide; chromic anhydride; chromic acid anhydride (Anger et al., 2005)	Chromic(VI) acid; chromium hydroxide oxide; dichromic acid ($H_2Cr_2O_7$)	Sodium chromate(VI); chromium disodium oxide; disodium chromate; rachromate; chromic acid, disodium salt; chromate of soda	Sodium dichromate(VI); sodium bichromate; dichromic acid, disodium salt; bichromate of soda	Dichromic acid, disodium salt, dihydrate
Structure (ChemID Plus, 2008)					
Molecular weight	99.994 (Lide, 2008)	118.010 (H_2CrO_4) (Lide, 2008); 218.001 ($H_2Cr_2O_7$) (ChemID Plus)	161.974 (Lide, 2008)	261.968 (Lide, 2008)	297.999 (Lide, 2008)
Molecular formula	CrO_3 (ChemID Plus, 2008)	H_2CrO_4; $H_2Cr_2O_7$ (ChemID Plus, 2008)	Na_2CrO_4 (ChemID Plus, 2008)	$Na_2Cr_2O_7$ (ChemID Plus, 2008)	$Na_2Cr_2O_7 \cdot 2H_2O$ (ChemID Plus, 2008)
Form	Dark red, deliquescent bipyramidal prismatic crystals, flakes, or granular powder (O'Neil, 2006)	Exists only as an aqueous solution (Lide, 2008); yellow to orange-red (Anger et al., 2005)	Yellow, orthorhombic crystals (Anger et al., 2005)	Light brown to orange-red plates (Anger et al., 2005)	Orange-red, monoclinic, translucent needles (Anger et al., 2005)
Stability/ reactivity	Deliquescent; decomposition begins above 198°C (Anger et al., 2005); powerful oxidizer (O'Neil, 2006)	Strong oxidizing agent (Anger et al., 2005)	Hygroscopic (Anger et al., 2005)	Strongly hygroscopic; decomposes above 400°C (Lide, 2008); strong oxidizing agent (Anger et al., 2005)	Very hygroscopic, deliquesces in air; decomposes above 85°C; strong oxidizing agent in acid solution (Lide, 2008; Anger et al., 2005)
Melting point	197°C (Lide, 2008)	Not applicable	794°C (Lide, 2008)	357°C (Lide, 2008)	Decomposes prior to melting (Lide, 2008)
Density	2.7 g/cm^3 (Lide, 2008)	Not applicable	2.72 g/cm^3 (Lide, 2008)	2.52 g/cm^3 (Anger et al., 2005)	2.35 g/cm^3 (Lide, 2008)
Water solubility	169 g/100 g H_2O at 25°C (Lide, 2008)	Not applicable	87.6 g/100 g H_2O at 25°C (Lide, 2008)	187 g/100 g H_2O at 25°C (Lide, 2008)	272.9 g/100 g H_2O (73.18 wt%) at 20°C (Anger et al., 2005)
Other solubility	Soluble in alcohol and mineral acids (Lewis, 2007)	Not applicable	Slightly soluble in ethanol (Lide, 2008)	Not available	Soluble in acetic acid (Lide, 2008)

Table 2-2. Physical properties of selected hexavalent chromium compounds

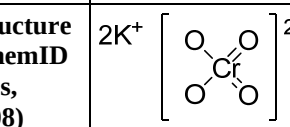
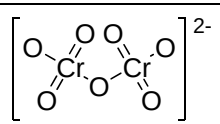
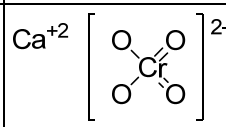
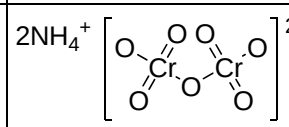
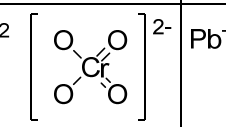
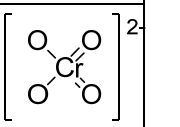
Name	Potassium chromate	Potassium dichromate	Calcium chromate	Ammonium dichromate	Zinc chromate	Lead chromate
CASRN	7789-00-6	7778-50-9	13765-19-0	7789-09-5	13530-65-9	7758-97-6
Synonyms (ChemID Plus, 2008)	Potassium chromate(VI); bipotassium chromate; dipotassium chromate; chromate of potash; tarapacaite; chromic acid, dipotassium salt	Potassium dichromate(VI); bichromate of potash; potassium bichromate; dipotassium bichromate; dipotassium dichromate; dipotassium dichromium heptaoxide; lopezite; dichromic acid, dipotassium salt	Calcium chromate(VI); calcium chrome yellow; calcium monochromate; gelbin; yellow ultramarine; chromic acid, calcium salt	Ammonium bichromate; ammonium dichromate(VI); diammonium dichromate; chromic acid, diammonium salt	Zinc chromate(VI) hydroxide; buttercup yellow; chromic acid, zinc salt; zinc chrome yellow; zinc teraoxychromate	Lead chromate(VI); phoenicochroite; plumbous chromate; chromic acid, lead salt; chrome yellow (O'Neil, 2006)
Structure (ChemID Plus, 2008)						
Molecular weight	194.191 (Lide, 2008)	294.185 (Lide, 2008)	156.07 (Lide, 2008)	252.065 (Lide, 2008)	181.403 (Lide, 2008)	323.2 (Lide, 2008)
Molecular formula	K_2CrO_4 (ChemID Plus, 2008)	$K_2Cr_2O_7$ (ChemID Plus, 2008)	$CaCrO_4$ (ChemID Plus, 2008)	$(NH_4)_2Cr_2O_7$ (ChemID Plus, 2008)	$ZnCrO_4$ (ChemID Plus, 2008)	$PbCrO_4$ (ChemID Plus, 2008)
Form	Lemon yellow prisms (Anger et al., 2005)	Tabular or prismatic, bright orange-red triclinic crystals (Anger et al., 2005)	Yellow monoclinic or rhombic crystals (O'Neil, 2006)	Large, bright, orange-red crystals (Anger et al., 2005)	Yellow prisms (Lide, 2008)	Yellow-orange monoclinic crystals (Lide, 2008)
Stability/ reactivity	Nonhygroscopic (Anger et al., 2005)	Nonhygroscopic; decomposes at 500°C (Anger et al., 2005; Lide, 2008)	Decomposes at 1,000°C (Lide, 2008); oxidizing agent (Lewis, 2007)	Flammable; nonhygroscopic; decomposition begins upon heating at 180°C (O'Neil, 2006). Strong oxidizing agent, may explode in contact with organic materials (Lewis, 2007)	Not available	Not available
Melting point	974°C (Lide, 2008)	398°C (Lide, 2008)	Decomposes prior to melting (Lide, 2008)	Decomposes prior to melting (Lide, 2008)	316°C (Lide, 2008)	844°C (Lide, 2008)
Density	2.73 g/cm^3 (Lide, 2008)	2.68 g/cm^3 (Lide, 2008)	3.12 g/cm^3 (Anger et al., 2005)	2.155 g/cm^3 (Lide, 2008)	3.40 g/cm^3 (Lide, 2008)	6.12 g/cm^3 (Lide, 2008)

Table 2-2. Physical properties of selected hexavalent chromium compounds

Name	Potassium chromate	Potassium dichromate	Calcium chromate	Ammonium dichromate	Zinc chromate	Lead chromate
Water solubility	65.0 g/100 g H_2O at 25°C (Lide, 2008)	15.1 g/100 g H_2O at 25°C (Lide, 2008)	4.5 g/100 g H_2O (4.3 wt%) at 0°C (Anger et al., 2005)	35.6 g/100 g H_2O at 20°C (Lide, 2008)	3.08 g/100 g H_2O (Lide, 2008)	0.000017 g/100 g H_2O at 20°C (Lide, 2008)
Other solubility	Insoluble in alcohol (O'Neil, 2006)	Insoluble in alcohol (Lewis, 2007)	Soluble in dilute acids; practically insoluble in alcohol (O'Neil, 2006)	Soluble in alcohol (Lewis, 2007)	Dissolves readily in acids (Anger et al., 2005); insoluble in acetone (Lide, 2008)	Insoluble in acetic acid; soluble in solutions of fixed alkali hydroxides; soluble in dilute nitric acid (O'Neil, 2006)

[a]Chromic acid is formed in aqueous solution when chromium(VI) oxide is dissolved in water; it cannot be isolated as a pure compound out of solution (Anger et al., 2005; Page and Loar, 2004). The term chromic acid is sometimes used in reference to chromium(VI) oxide; however, it should be noted that there is a structural difference between the anhydrous substance chromium(VI) oxide and the aqueous chromic acid that forms when the oxide is dissolved in water.
[b]Chromic acid exists in solution as both H_2CrO_4 and $H_2Cr_2O_7$ (Anger et al., 2005; Page and Loar, 2004; Cotton et al., 1999). H_2CrO_4 is the main species in basic solutions (pH >6) while $H_2Cr_2O_7$ is the main species in strongly acidic solutions (pH <1) (Anger et al., 2005; Page and Loar, 2004; Cotton et al., 1999). Both species are present in equilibrium in solutions that have a pH value between 2 and 6 (Anger et al., 2005; Page and Loar, 2004; Cotton et al., 1999).

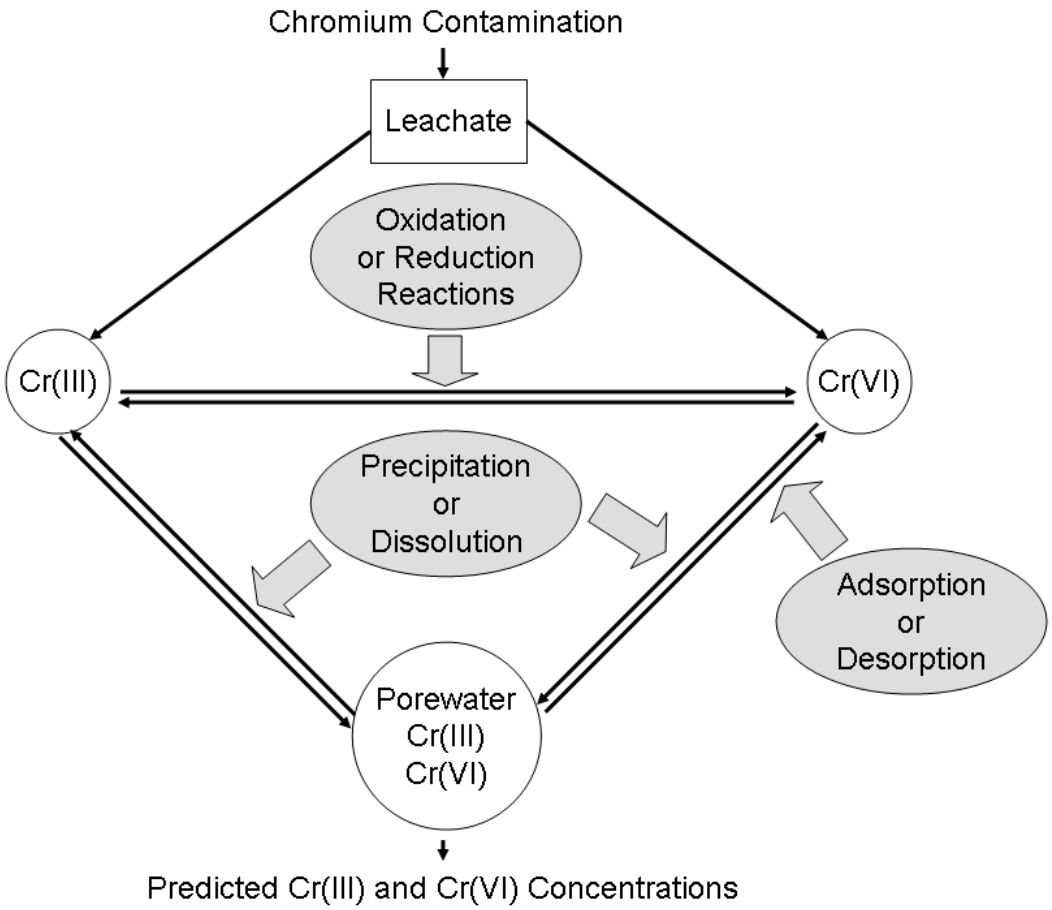
Table 2-3. Standard potentials for oxidation-reduction equilibria among chromium valence states

Half-cell reaction	E^0 (V)	Change
$Cr_2O_7^{2-} + H_2O + 2e^- \rightarrow 2CrO_4^{3-} + 2H^+$	0.55	VI to V
$Cr_2O_7^{2-} + 6H^+ + 4e^- \rightarrow 2CrO_2 + 3H_2O$	0.95	VI to IV
$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$	1.38	VI to III
$CrO_4^{3-} + 4H^+ + e^- \rightarrow CrO_2 + 2H_2O$	1.34	V to IV
$CrO_4^{3-} + 8H^+ + 2e^- \rightarrow Cr^{3+} + 4H_2O$	1.72	V to III
$CrO_2 + 4H^+ + e^- \rightarrow Cr^{3+} + 2H_2O$	2.10	IV to III
$Cr^{3+} + e^- \rightarrow Cr^{2+}$	-0.42	III to II
$Cr^{3+} + 3e^- \rightarrow Cr$	-0.74	III to 0
$Cr^{2+} + 2e^- \rightarrow Cr$	-0.90	II to 0
$CrO_4^{2-} + 4H_2O + 3e^- \rightarrow [Cr(OH)_4]^- + 4OH^-$	-0.72	VI to III
$CrO_4^{2-} + 4H_2O + 3e^- \rightarrow Cr(OH)_3 + 5OH^-$	-0.11	VI to III
$[Cr(OH)_4]^- + 3e^- \rightarrow Cr + 4OH^-$	-1.33	III to 0
$Cr(OH)_3 + 3e^- \rightarrow Cr + 3OH^-$	-1.33	III to 0

Source: Emsley (1989) as cited in Katz and Salem (1993).

The environmental chemistry of chromium is complex. Figure 2-2 illustrates the possible fates of chromium in soil/water systems. Chromium is known to undergo various chemical and biological reactions in natural systems that govern its speciation, and in turn, environmental behavior. Important reactions include oxidation/reduction, precipation/dissolution, and adsorption/desorption. Both oxidation of Cr(III) and reduction of Cr(VI) can occur in geologic and aquatic environments. Hexavalent chromium is a strong oxidizing agent and is readily reduced in the presence of appropriate electron donors as shown here:

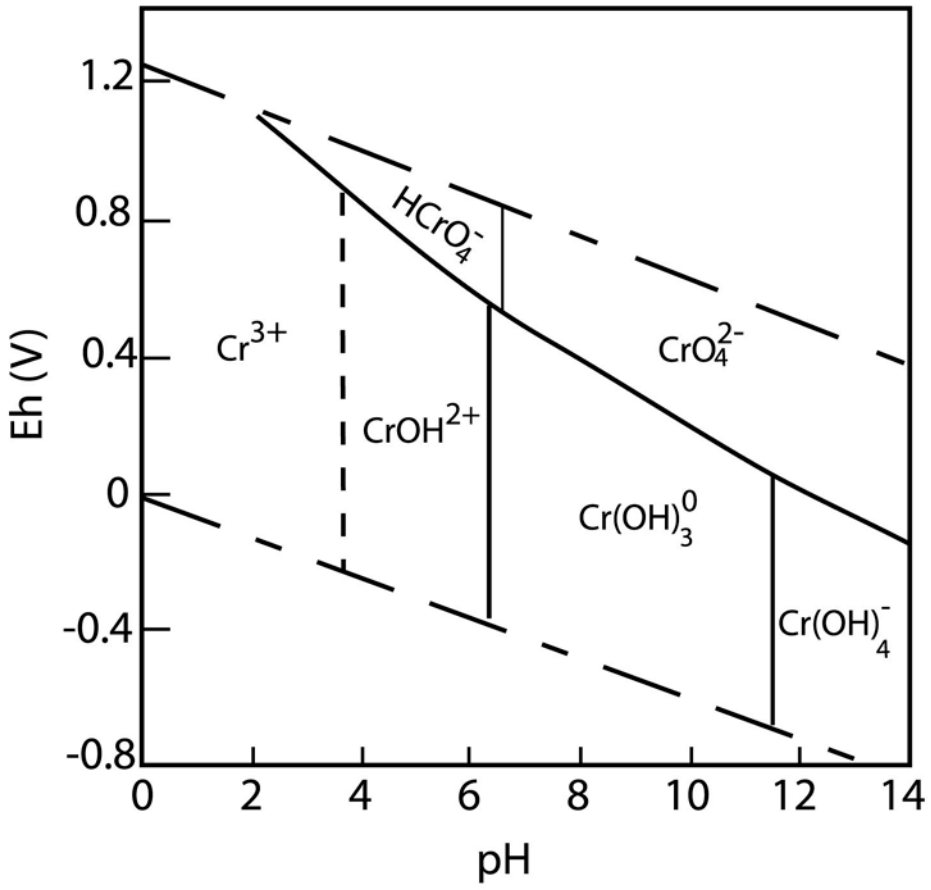
$$HCrO_4^- + 7H^+ + 3e^- \Leftrightarrow Cr^{3+} + 4H_2O \qquad (2\text{-}1)$$



Source: Adapted from Rai et al. (1989).

Figure 2-2. Schematic of possible reaction processes (grey ovals) that determine disposition and speciation of chromium contamination in the environment.

Chromium is a redox active soil contaminant with dramatic alterations in its mobility and toxicity with changes in oxidation state (Fendorf et al., 2004; Rai et al., 1989). Chromium can exist in oxidation states ranging from -2 to +6, but only +3 and +6 are typically found within the range of pH and redox potential common in environmental systems (Shupack, 1991). Figure 2-3 depicts a generalized scheme of equilibrium potentials versus pH for chromium.



Source: Rai et al. (1989).

Figure 2-3. Stability diagram showing aqueous speciation of chromium at various equilibrium potential (Eh, volts) and pH.

Hexavalent chromium is a strong oxidizer and as a result, exists only in oxygenated species that are very soluble and pH-dependent according to the following equilibria (Nieboer and Jusys, 1988 as cited in Losi et al., 1994):

$$H_2CrO_4 \Leftrightarrow H^+ + HCrO_4^- K_{a1} = 10^{0.6} \quad (2\text{-}2)$$

$$HCrO_4^- \Leftrightarrow H^+ + CrO_4^{2-} K_{a2} = 10^{-5.9} \quad (2\text{-}3)$$

Neuss and Rieman (YEAR, as cited in Katz and Salem, 1993) evaluated the acid functions and arrived at K_{a1} and K_{a2} values of 1.8×10^{-1} and 3.2×10^{-7}. From equations (2-2) and (2-3) it can be observed that at very low pH values (pH < 1), H_2CrO_4 is the predominant species, while between pH 0 and 5.9, the $HCrO_4^-$ and $Cr_2O_7^{2-}$ anions prevail (Shupack, 1991). At pH 6 or above, CrO_4^{2-} prevails. Thus, H_2CrO_4 and CrO_4^{2-} should be most abundant in natural systems.

Further, at concentrations greater than 0.01M (520 mg/L) dimerization of the chromate ion occurs which yields the dichromate ion (Whitten and Gailey, 1984 as cited in Losi et al., 1994):

$$2CrO_4^{-} + 2H^{+} \Leftrightarrow Cr_2O_7^{2-} + H_2O \quad K_c = 10^{14.6} \tag{2-4}$$

$$\frac{\left[Cr_2O_7^{2-}\right]}{\left[CrO_4^{2-}\right]^2\left[H^{+}\right]^2} = 10^{14.6} \tag{2-5}$$

This reaction is pH sensitive as well, with dichromate favored at lower pH (Losi et al., 1994). Solving equation (2-4) using values for chromate concentration and pH that would likely be encountered in contaminated groundwater (5.2 mg/L and 7.0), the ratio of dichromate to chromate would be 0.04. Thus, hexavalent chromium chemistry in environmental systems is largely confined to that of the chromate anion.

Speciation of hexavalent (CrVI) and trivalent (CrIII) chromium will generally depend on a variety of environmental parameters including: pH, concentration, and the ligands available in the matrices (Katz, 1991). In most natural systems, Cr(VI) will be present as CrO_4^{2-} and major trivalent species may include hydroxides and various organic complexes (Losi et al., 1994). The acid anhydride CrO_3 and acid chloride CrO_2Cl_2, and a wide variety of metal chromates $MCrO_4$ and metal dichromates MCr_2O_7 are typical hexavalant compounds (Katz and Salem, 1993). Chromium(VI) may also form other species, including $HCr_2O_7^{-}$ and CrO_4^{2-}, but their formation requires Cr(VI) concentrations $>10^{-2}$ M, which are not found commonly in natural waters (Baes and Mesmer, 1986 as cited in Rai et al., 1989).

Natural occurrence of hexavalent chromium is rare as it is readily reduced by organic matter in the environment (Ashley et al., 2003; Barceloux, 1999; U.S. EPA, 1984). Industrial releases of hexavalent chromium compounds to surface water and soil can result in the transport and leaching of these substances into groundwater, provided these substances remain under oxidizing conditions (Loyuax-Lawniczak et al., 2001; Pellerin and Booker, 2000; James et al., 1997). Hexavalent chromium compounds released to the environment by anthropogenic sources may persist in natural waters and soils that contain low amounts of organic matter (Johnson et al., 2006; Loyaux-Lawniczak et al., 2001; U.S. EPA, 1984). Hexavalent chromium compounds are considered to be more soluble in water and have greater mobility in soil than other types of chromium compounds (Loyuax-Lawniczak et al., 2001; James et al., 1997).

Whereas reduction of Cr(VI) is likely to occur in environmental systems, oxidation of Cr(III) is not frequently observed with the exception of some oxidation in the presence of Mn^{4+} (Losi et al., 1994). Thus, Cr(III) is considered more stable in most natural systems. Trivalent

chromium is also less mobile than Cr(VI) in most soil/water systems due to its relative insolubility at relevant pH values (>5).

Consideration of these environmental chemistry factors determining speciation, solubility, and mobility are of critical importance in assessing potential environmental hazards, relevance of available risk estimates, and remediation strategies for sites with high levels of chromium. The behavior of both hexavalent and trivalent chromium and the interconversion between these forms must be understood when attempting to characterize bioaccessibility and environmental contamination with chromium. Further, accurate data for describing the dominant reactions in each process must be available for characterization of a particular site (Rai et al., 1989).

2.3. ANALYTICAL METHODS

Analysis of chromium in either environmental or biological samples must consider the thermodynamic stability of each oxidation state as a function of pH and the kind of species, i.e., the anions, cations, and polymeric ions that can form for each oxidation state as a function of the sample such as pH, lability, equilibria, temperature, and the presence of other oxidants and reductants (Katz, 1991; Shupack, 1991). Metal speciation is important because the biogeochemical behavior, environmental mobility, bioaccessibility, bioavailability, and subsequent toxicity all depend on it (Fytianos, 2001). Thus, the level of detection and the ability to detect speciation versus total chrome in various environmental and biological compartments is a critical consideration when evaluating the relevance and reliability of any given environmental or toxicological data. While the topic is discussed briefly in this section with respect to environmental bioaccessibility, it is equally relevant to discussions on bioavailability and to considerations of experimental design in the toxicological studies in subsequent sections. Attention should also be paid to the limit of detection (LOD) for the analytical method used.

Due to its redox sensitivity described above, any attempt at analysis of samples of soil and water for chromium content should address speciation as a goal, especially due to the differences in toxicity between Cr(VI) and Cr(III) compounds. Critical considerations include sample treatment and storage, extraction, and preparation in the case of soils, and the actual analytical method used. Analysis of chromium in soils and sediments is a special challenge. It is important that samples be field-moist, sieved (4 mm), well-mixed and stored at 4°C (Bartlett, 1991 as cited in Losi et al., 1994). Quality assurance and quality control exercises are also recommended (Katz, 1991).

Approximate limits of detection for several analytical methods commonly used for chromium are provided in Table 2-4. While quantification of chromium in certain biological compartments may require extremely sensitive detection methodology (see below), analysis of chromium in environmental samples is typically done by either atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP) spectrometry for total chromium, and colorimetry

(diphenylcarbazide method) and high-performance liquid chromatography (HPLC) for quantifying Cr(VI). The difference between the total chromium and Cr(VI) measurements is assumed to be Cr(III). All of these methods are subject to interferences that must be evaluated through the use of appropriate matrix spikes. Comparative discussions across studies must take into account differences in analytical LOD and speciation. Further, translation of dose-response estimates based on speciated chromium concentrations must be rectified with the sampling methods used for exposure assessment as part of risk characterization and management efforts. Vitale et al. (1997) have reviewed the challenges of chromium speciation as it relates to several of the most current recommended EPA methods, including EPA Method 218.6 for water and SW-846 methods for soils, sediments, and wastes.

Table 2-4. Detection limits for methods commonly used in the analysis of chromium in water and soil extracts

Method	Species detected	Approximate detection limit (µg/L)
Flame atomic absorption spectrometry	Total chromium	500[a]
Graphite furnace atomic absorption spectrometry	Total chromium	1[a]
Inductively coupled plasma mass spectrometry	Total chromium	6–10[a]
Colorimetric (diphenylcarbazide)	Cr(VI)	50[b]
High-performance liquid chromatography (single-column ion-chromatography	Cr(VI)	92[c]

[a]Bartlett (YEAR), see Gochfeld (1991)
[b]Mehra and Frankenberger (1989)
[c]MISSING

Source: Losi et al. (1994).

Chromium in biological samples traditionally has been determined by graphite furnace atomic absorption spectrometry, neutron activation analysis, or inductively coupled plasma optical emission spectrometry (ICP-OES). More recently, inductively coupled plasma mass spectrometry (ICP-MS) has become popular as a method due to its low detection limits and multi-element analysis capabilities. Levine et al. (2007) developed and validated an ICP-MS method after a rapid, open-vessel microwave digestion, to determine total chromium in the tissues of the F344 rats used in the National Toxicology Program (NTP) studies of orally ingested sodium dichromate dihydrate and chromium picolinate monohydrate described in (see Sections 4 and 5). Performance of the method was evaluated using kidney tissue across a range of 0.50 to 5.00 µg Cr/g tissue. Feces samples were analyzed by ICP-OES because of the relatively high levels of chromium in this matrix (Levine et al., 2007). Matrix recovery for the ICP-MS method ranged from 76.6 to 103%; mean recovery was 97.4%. Percent relative standard deviations for both intra- and inter-assay preparations ranged from 0.88 to 13%. The LOD, calculated as three times the SD of these matrix samples, was determined to be 0.01 ng/L,

equivalent to 0.02 µg Cr/g kidney tissue. Cross-validation with $B6C3F_1$ mouse kidney tissue was also demonstrated.

Because a majority of absorbed chromium is excreted in the urine, occupational biomonitoring of urinary concentrations of water soluble chromium compounds (as total chromium) has been successfully used to assess whether significant inhalation exposure to chromium has occurred (ACGIH, 2004). The New Jersey Department of Health (NJDOH) has also suggested that urinary chromium concentrations might serve as a useful indicator of environmental exposures, but an expert panel cited concerns for differentiating environmental contributions from dietary sources (Anderson et al., 1993). Food known to contain moderate levels of Cr(III) include breads, cereals, spices, fresh vegetables, meat, fish, vitamin supplements, Brewer's yeast, and beer (Gargas et al., 1994), and although comparison of urinary chromium concentration to the administered dose (exposure) provides an estimate of the amount of chromium systemically absorbed, it does not provide an indication of the valence state in which it was absorbed. Therefore, elevated urinary chromium levels should be carefully considered and may be misleading if interpreted as a biomarker for Cr(VI) exposure or toxicity (Kerger et al., 1996a). Typical background urinary chromium concentrations vary from ≈0.24 to 1.8 µg Cr/L in healthy individuals (IARC, 1990).

Atomic absorption spectrometry with a graphite furnace is currently recommended for urinary biomonitoring programs (Paustenbach et al., 1997). Guidelines developed by Veillon et al. (1982) show that a LOD of 0.05 µg/L is achievable for undiluted samples using this method, whereas commercial laboratories typically report a LOD of 0.2 µg/L. As with other biomonitoring evaluations, controlling for confounding variables is essential to be able to understand their correlation with physiological effects. Confounding variables specifically associated with elevated urinary chromium levels include diet, regular exercise, smoking habits, beer consumption, past employment in chromium-related occupations, and health status (Paustenbach et al., 1997). Pregnancy and stress have been shown to enhance losses of chromium (Anderson et al., 1989), and diabetics typically show chromium levels twofold higher than nondiabetics (Bukowski et al., 1991).

In addition to urinary biomonitoring, other biological matrices have been used to measure recent chromium exposures, including whole blood, plasma, and hair (IARC, 1990). Concentration of total chromium in RBCs is considered to be a more specific biomarker for characterizing recent exposure to Cr(VI) (Korallus, 1986; Lewalter et al., 1985). The biochemistry of chromium inside cells is discussed in Section 3.

Factors to be considered when implementing a monitoring program or when evaluating results of studies using these matrices include the species of exposure, duration and dose of exposure, and the analytical techniques and achievable LOD. Advantages and disadvantages are associated with measuring chromium in each matrix and should be considered carefully, especially when evaluating the dose-response behavior of different exposures and dosing

regimens across various toxicological studies. Negative and positive attributes of each biomonitoring technique are provided in Table 2-5.

Table 2-5. Biomonitoring options for assessing chromium exposure

Biological matrix	Advantages	Disadvantages
Urine	Easy sample collection, noninvasive, can evaluate high-level recent (within 48-hour) occupational exposure, good correlation for inhalation exposures[a]	Samples easily contaminated, difficulty in distinguishing Cr levels from background, analytical limit of detection of 0.2 µg/L (typical background range), inability to distinguish between Cr(III) and Cr(VI) exposure, seriously affected by confounding variables[b]
Red blood cell (RBC)	Chromium detected up to 120 days (lifetime of RBC) following exposure, Cr(III) and Cr(VI) can be differentiated, analytical limit of detection of 0.09 µg/L blood, good correlation for Cr(VI) oral and inhalation exposures;[c] Cr(VI) exposures may be identified 120 days post exposure.	Invasive technique, trained professionals required to collect samples, collection and analysis without contamination are difficult, costly to study large populations
White blood cell (WBC)	Animal studies show good correlation to Cr(VI) via oral and intravenous (i.v.) exposure, accumulates Cr(V) exclusively, accumulates Cr(VI) to a greater extent than RBC[d]	Does not accumulate Cr(III), invasive technique, trained professionals required to collect samples, difficult to study large populations
Plasma	Only Cr(III) confined to plasma compartment[e]	Cr(VI) only detected up to 2 hours following exposure, reduction of Cr(VI) in plasma is significant, invasive technique, trained professionals required to collect samples, difficult to study large populations[e]
Hair	Easy sample collection, noninvasive, occupational exposure to Cr(VI) [in the absence of Cr(III)] via inhalation has been correlated[f]	Can not distinguish between Cr(III) and Cr(VI) exposure, inability to correlate time of exposure[f]

[a]Mutti et al. (1979) and Tola et al. (1977) as cited in Paustenbach et al. (1997); [b]Anderson (1983), Gargas et al. (1994), and Wiegand et al. (1988) as cited in Paustenbach et al. (1997); [c]Gray and Sterling (1950), Wiegand et al. (1988), and Kerger et al. (1996) as cited in Paustenbach et al. (1997); [d]Coogan et al. (1991) as cited in Paustenbach et al. (1997); [e]Wiegand et al. (1988) as cited in Paustenbach et al. (1997); [f]Saner et al. (1984) as cited in Paustenbach et al. (1997).

Source: Paustenbach et al. (1997)

3. TOXICOKINETICS

The internal environment of the GI tract, similar to the external environment discussed in Section 2, can also affect the digestion, solubilization, and speciation of chromium compounds, and thus impact their internal bioaccessibility (defined as the ability of chromium to be released from the environmental matrix to which it is bound, i.e., the fraction of the dose ingested that becomes freely available for absorption via crossing a cellular membrane), bioavailability (defined as the potential for chromium to cross cellular boundaries, i.e., the degree to which it becomes available to the target tissue after administration), and toxicokinetics within the body. This section of the toxicological review discusses key determinants of these internal processes, and why insights into how these processes vary across species and tissues are a critical consideration for the mode of carcinogenic action of hexavalent chromium, Cr(VI).

As depicted in Figure 2-1 in the previous section, both environmental and internal bioaccessibility processes are extracellular. Section 3.1 will discuss those physiological processes, internal to the organism (but still extracellular), that impact bioaccessibility. Bioavailability (processes D and E in Figure 2-1) will be discussed in Section 3.2. The available data on the biochemistry of intracellular reactions involving chromium will be summarized in Section 3.3. In Section 3.4, the impact of bioaccessibility and bioavailability on the toxicokinetic component of the cancer mode of action for Cr(VI) will be discussed. These toxicokinetic considerations are an important aspect of the mode of action and provide insights into the evaluation of the tissue responses discussed in Section 4. Section 3.5 includes an evaluation and discussion of available model structures to describe internal dosimetry of Cr(VI). Finally, considerations of chromium essentiality versus toxicity are discussed in Section 3.6.

3.1. BIOACCESSIBILITY OF INGESTED HEXAVALENT CHROMIUM

3.1.1. In Vitro and Ex Vivo Studies of Bioaccessibility

A variety of in vitro and ex vivo studies have attempted to estimate the extent of reduction of Cr(VI) to Cr(III) that is achieved in the GI lumen. The in vitro studies typically develop surrogate formulations of physiological fluids, such as gastric juices, or employ different beverages or diets in order to evaluate their reductive potential. Ex vivo studies of bioaccessibility, on the other hand, evaluate the reductive potential of actual samples of saliva or gastric juices.

Gammelgaard et al. (1999) used an artificial gastric juice to evaluate the degree of reduction of Cr(III) inorganic compounds, chromium(III) picolinate, and hexavalent chromium, Cr(VI). The artificial gastric juice was prepared from 2.0 g sodium chloride and 3.2 g pepsin (1:10,000) dissolved in 4 ml of concentrated hydrochloric acid, diluted to 1 L with Milli-q water with the pH adjusted to 1.2 with hydrochloric acid (HCl). All analyses were performed in

duplicate using atomic absorption spectrometry. The vessel volume was 1 L and the surrounding water bath was kept constant at 37 ± 0.5° C. A volume of 900 ml of this artificial gastric juice was added to potassium dichromate for an initial concentration of 100 µg/L and the Cr(VI) concentration was observed for 4 hours. Chromium picolinate at a concentration of 100 µg/L was studied in a similar fashion; experiments were repeated six times. Simultaneous determination of Cr(VI) and Cr(III) was performed by ion chromatography with chemiluminescence; the detection limit for both Cr(III) and Cr(VI) was 0.1 µg/L. The conversion of Cr(VI) to Cr(III) in gastric juice followed first-order kinetics and a half-life ($t_{1/2}$) of 23 minutes was calculated. In contrast, the organic chromium picolinate was unchanged.

Donaldson and Barreras (1966) performed ex vivo studies using everted sections of female albino rat intestine. Post sacrifice, the intestines were immediately lavaged in situ with saline, everted on glass rods, and cut into 1/8-inch sections. Rings from the proximal half of the bowel were randomly distributed in Erlenmeyer flasks containing a range of concentrations (0.1 to 5.0 µg/ml) of either trivalent $Cr^{51}Cl_3$ or hexavalent $Na_2Cr^{51}O_4$ in 10 ml of Krebs-Ringer bicarbonate solution. Incubations were conducted for 60 minutes at 95%O_2:5%CO_2. Tissues were then rinsed and digested with H_2SO_4 prior to assay for radioactivity. In vitro intestinal uptake of the hexavalent $Na_2Cr^{51}O_4$ was linear over the concentration range and significantly greater than uptake of the trivalent $Cr^{51}Cl_3$.

The effect of gastric juice composition on uptake in these GI tissue samples was investigated by mixing 25 µg of the labeled chromium compounds with 8 ml of either 0.1 N HCl, human gastric juice (pH 1.4), or previously neutralized gastric juice (Donaldson and Barrera, 1966). After 30 minutes of incubation at room temperature, these mixtures were neutralized (to pH 7.0) with 0.1 N NaOH and diluted to 25 ml with NaCl. Everted intestinal rings were then incubated as described previously with 10 ml of the neutralized mixture and 1 µg/l of either labeled chromium compound. To determine the proportion of the radioactivity bound to gastric juice macromolecules, duplicate 5-ml aliquots were dialyzed in Viking cellulose bags against repeated changes of NaCl for 48 hours. Results for these studies are shown in Table 3-1.

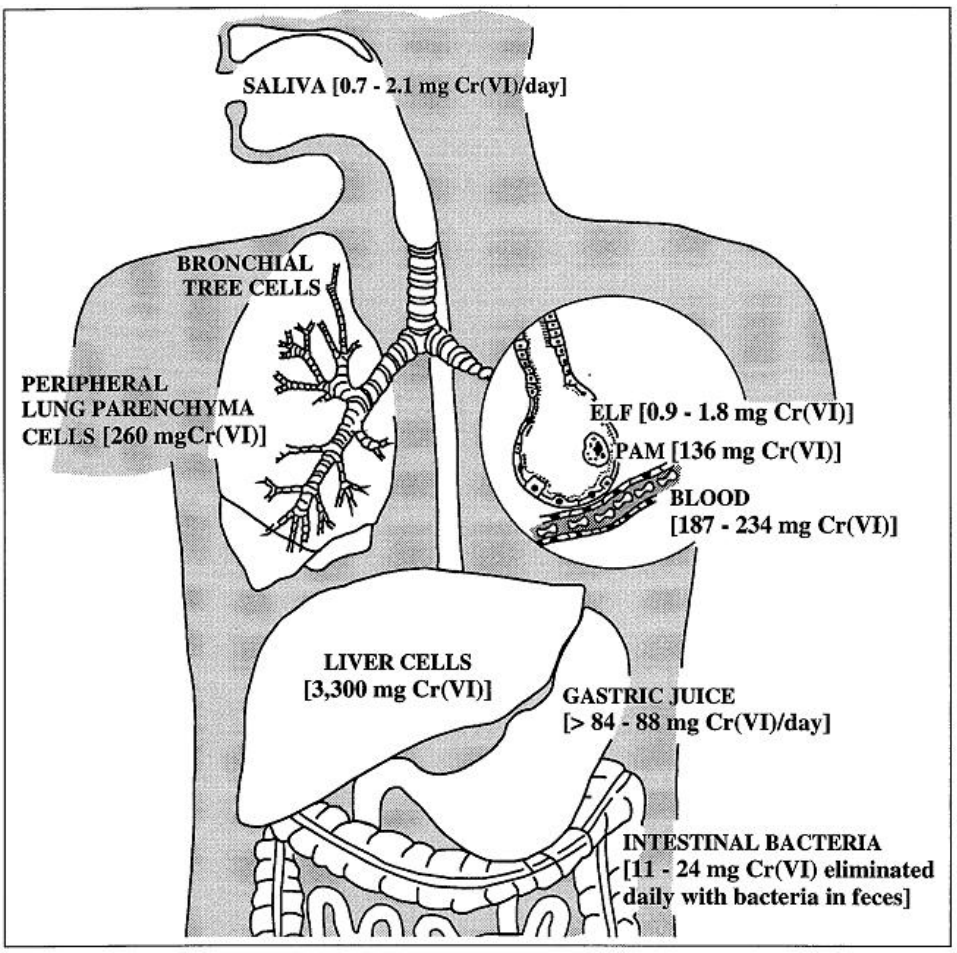
Table 3-1. Effect of gastric juice composition on binding and in vitro uptake by rat intestinal rings of trivalent ($Cr^{51}Cl_3$) or hexavalent ($Na_2Cr^{51}O_4$) radiolabeled chromium compounds

Tissue preparation	Binding by gastric juice (µg/ml)[1]	Uptake by intestinal rings (µg/gm)[1]
Trivalent $Cr^{51}Cl_3$ without gastric juice	–	0.9 ± 0.1
Plus gastric juice pH 7.0	1.8 ± 0.2	0.2 ± 0.1
Plus gastric juice pH 1.4	2.0 ± 0.2	0.2 ± 0.1
Hexavalent $Na_2Cr^{51}O_4$ without gastric juice	–	2.7 ± 0.4
Plus gastric juice pH 7.0	0.0 ± 0.0	2.5 ± 0.3
Plus gastric juice pH 1.4	1.4 ± 0.2	0.8 ± 0.3

[1]Mean result of 4 experiments ± SD

Source: Donaldson and Barrera (1966).

DeFlora and collaborators (DeFlora et al., 1997; DeFlora and Wetterhahn, 1989; De Flora et al., 1987; Petrilli and DeFlora, 1982) performed a series of studies to estimate the ability of various human physiological fluids and tissues to reduce or sequester Cr(VI). The summary data shown in Table 3-2 and depicted in Figure 3-1 represent a synthesis of data from studies in that laboratory with anatomical and physiological parameters as described in detail in the source reference (DeFlora et al., 1997). These parameters were used to arrive at estimates of the overall Cr(VI) reducing or sequestering capacity of human body compartments relative to oral and inhalation exposures. The general term "sequestration" was used to connote when intact cells were tested and the term "reduction" was used when cell homogenates or their subfractions were tested in the presence of an exogenous NADPH-generating system, an S9 mix. Estimates of overall Cr(VI) reducing or sequestering capacity were calculated by multiplying the specific reducing activity of a given organ, cell population, or fluid expressed as µg Cr(VI) reduced per unit of weight, volume, or number, by the average content of the same organ, cell population, or fluid in the human body (De Flora et al., 1997). De Flora (2000) proposed that these reduction capacities account for the limited toxicity for Cr(VI) after oral ingestion due to efficient detoxication by saliva, gastric juice and intestinal bacteria; similarly, lung cancer is only induced when Cr(VI) doses overwhelm the reductive capacity of the fluid of the epithelial lining, pulmonary alveolar macrophages, and bronchial tree and peripheral lung parenchyma cells. De Flora (2000) also suggested that efficient uptake and reduction of Cr(VI) in RBC explains the lack of carcinogenicity at sites remote to the portal of entry.



Source: De Flora et al. (1997).

Figure 3-1. Estimates of Cr(VI) sequestration or reduction by organs, cell populations and fluids in the human body relevant to portal of entry uptake or effects on remote distribution kinetics. See Table 3-2 and text for details on calculations.

Table 3-2. Estimates of the overall chromium(VI) reducing capacity of human fluids, cells and tissues

Organ, cell population, or body fluid	Weight of organs, number/weight/volume of cells, or volume of body fluid	Chromium(VI) reduction or sequestration (mean ± SD)	Overall chromium(VI) reducing or sequestering capacity per individual
Saliva	500–1500 ml/day	1.4 ± 0.2 µg/ml	0.7–2.1 mg/day
Gastric juice	1000–15000 ml/day (fasting) + 800 ml/meal 3400–3900 ml/day (3 meals)	8.3 ± 4.3 µg/ml 31.4 ± 6.7 µg/ml	8.3–12.5 mg/day during interdigestive periods + 25.1 mg/meal 84–88 mg/day (3 meals)
Intestinal bacteria	2.9–6.3 g eliminated daily with feces	3.8 ± 1.7 µg/10^9 bacteria	11–24 mg eliminated daily with feces
Liver	1500 g	2.2 ± 0.9 mg/g liver homogenate	3300 mg
Whole blood	4490 ml (males) 3600 ml (females)	52.1 ± 5.9 µg/ml intact blood	234 mg (males) 187 mg (females)
Red blood cells (RBC)	2030 ml (males) 1470 (females)	63.4 ± 8.1 µg/ml RBC lysate soluble fraction	128 mg (males) 93 mg (females)
Epithelial lining fluid (ELF)	37.5–75 ml	23.7 ± 15.9 µg/ml	0.9–1.8 mg
Pulmonary alveolar macrophages (PAM)	23 x 10^9 PAM	4.4 ± 3.9 µg/10^6 PAM S9 fraction	136 mg
Peripheral lung parenchyma	1300 g	0.2 ± 0.07 mg/g lung S12 fraction	260 mg

Source: De Flora et al. (1997).

It should be noted, however, that because of the ability of many organic molecules to reduce Cr(VI) in highly acidic solutions, the values reported by DeFlora and colleagues (DeFlora et al., 1997; DeFlora, 2000) should be considered with some caution. The DeFlora studies relied on the direct measurements of residual Cr(VI) in the calorimetric reaction with diphenylcarbazide in the presence of 8% sulfuric acid, conditions that likely overestimated the reducing capacities of these biological systems (Zhitkovich, 2005). More accurate determinations of Cr(VI) reductive activities require the removal of organic molecules by charcoal or other means prior to the addition of the reagent.

3.1.2. In Vivo Studies of Bioaccessibility

Donaldson and Barreras (1966) also performed in vivo studies of bioaccessibility in both female albino rats and human volunteers. Female albino rats were fasted overnight and then administered 1 ng of either radiolabeled trivalent $Cr^{51}Cl_3$ or hexavalent $Na_2Cr^{51}O_4$ in 1 ml 0.9% saline with an internal standard spike. Feces and urine were collected separately in individual metabolic cages for 7 days post dosing. Excretion was expressed as percentage of administered activity. Two weeks later, 1 ng of either radiolabeled trivalent $Cr^{51}Cl_3$ or hexavalent $Na_2Cr^{51}O_4$ in 1 ml 0.9% saline was injected in anesthesized rats through a laparoscopic incision into the lumen of the jejunem near the ligament of Treitz. The intestine proximal to the incision site was compressed to prevent retrograde flow of the fluid. Rats were allowed to recover and urine and feces collected as previously described. Fecal recovery of both forms of chromium was nearly complete after oral administration in water (98 ± 4.2%, for $Cr^{51}Cl_3$ and 97.7 ± 2.5% for $Na_2Cr^{51}O_4$); whereas urinary recovery was 1.4 ± 0.7% and 0.8 ± 0.4% for $Cr^{51}Cl_3$ and $Na_2Cr^{51}O_4$. Intrajejunal administration resulted in significant absorption, as indicated by increased urinary and decreased fecal recoveries of 16.5 ± 5.6% and 76.4 ± 8.9%, for the hexavalent $Na_2Cr^{51}O_4$, whereas only a slight increase in absorption for the trivalent $Cr^{51}Cl_3$ (91.6 ± 3.4 % in feces and 4.3 ± 1.7%) occurred.

Donaldson and Barreras (1966) reported that adult volunteers hospitalized for either obesity or pernicious anemia were administered 20 ng of trivalent $Cr^{51}Cl_3$ or hexavalent $Na_2Cr^{51}O_4$ either orally in drinking water or by an intestinal perfusion technique. The obesity volunteers were otherwise healthy and the pernicious anemia patients were not anemic, but still had histamine-fast achlorydria and vitamin B12 malabsorption when studied. For the ingestion studies, urine was collected for 24 hour. The injection tubing was inserted under fluoroscopic control into the small intestine so that its lumen opened at the ligament of Treitz as in the rats. The proximal collection tube was located 15 cm distal to that site, and the distal collection tube was 45 cm beyond. The infusion was performed at a constant rate of 10 ml/min after a 30-minute equilibration period. The perfusion fluid contained 1% polyethylene glycol (PEG) as a "nonabsorbable" reference marker and 2.5% xylose for calculation of absorption along with the administered chromium. Fluid was collected for 1 hour and then fresh fluid was used for a 2nd

1-hour collection period. Fecal samples were collected for 6 days in a single container in which the sample was homogenized. The majority of radioactivity was recovered in the feces when either the trivalent or hexavalent compound was administered orally, but absorption was slighty greater for the hexavalent form with recovery at 99.6 ± 1.8% for $Cr^{51}Cl_3$ and 89.4 ± 2.6% for $Na_2Cr^{51}O_4$. Urinary excretion was also slightly higher for the hexavalent $Na_2Cr^{51}O_4$ (2.1 versus 1.5%). After intraduodenal administration, fecal and urinary excretion again indicated a low absorption of the trivalent $Cr^{51}Cl_3$, whereas approximately 50% of the hexavalent $Na_2Cr^{51}O_4$ appeared to be absorbed based on fecal excretion and approximately 10% appeared in the urine. Calculation of the absorption using PEG confirmed a high absorption for the hexavalent chromium compound and a minimal amount for the trivalent chromium compound.

In an extension of this perfusion study using $Na_2Cr^{51}O_4$, Donaldson and Barreras (1966) incubated the perfusion fluid with 0.1 N HCL for 30 minutes prior to neutralization with 0.1 N NaOH and then used this fluid in the procedure described above with another five subjects. This treatment did not result in impairment of absorption. They then incubated $Na_2Cr^{51}O_4$ with 10 ml of gastric juice at pH 1.4 for 30 minutes and proceeded with the procedure as before. This pretreatment with gastric juice dramatically decreased the absorption to almost complete inhibition. Absorption of xylose remained constant in these studies. Since it appeared that absorption was significantly decreased by exposure to gastric juice, the researchers stratified their analysis to evaluate control subjects versus those with pernicious anemia, and found a significant decrease in the absorption for the latter group demonstrated by decreased fecal excretion and increased urinary excretion.

3.2. BIOVAILABILITY, DISPOSITION, AND ELIMINATION OF INGESTED HEXAVALENT CHROMIUM

The processes and factors that determine the ability of chromium to cross cellular boundaries is defined as bioavailability. Key mechanisms of bioavailability that determine internal tissue dose include the following: uptake through cell membranes, intracellular distribution, and binding to cellular macromolecules. The potential for a chromium compound to cross cellular boundaries is primarily affected by its solubility and valence state, which are, as with environmental chemistry of chromium described in Section 2, dependent upon solubility and pH. This section will describe the key events of chromium uptake and biochemistry within cells as critical to its subsequent disposition in and elimination from the body. Studies that provide a general understanding of the bioavailability and toxicokinetics of ingested Cr(VI) will first be discussed here.

The extent of absorption of ingested hexavalent chromium from the GI tract is determined by both the solubility of the hexavalent chromium compound ingested and how rapidly hexavalent chromium is reduced to trivalent chromium, as trivalent chromium does not diffuse as readily across cell membranes. Hexavalent chromium, on the other hand, can easily

cross cell membranes due to its ability to use existing nonspecific sulfate and phosphate anion transport mechanisms.

Generally, absorbed hexavalent chromium is distributed throughout the body, but the blood, liver, kidney, and spleen are the primary sites of distribution in addition to either the respiratory or GI tract as the portal of entry. Bone is also a site of distribution, which may contribute to the long-term retention kinetics of chromium. Absorbed chromium can be transferred to fetuses through the placenta and to infants via breast milk. Chromium can also be eliminated in hair, nails, and breast milk. There does not appear to be a gender difference in the toxicokinetics of hexavalent chromium, and inter-individual variability in extracellular reduction and subsequent absorption and elimination may be primarily driven by differences in gastric contents and intervals between meals.

Quantitative descriptions of the pathways and mechanisms for this distribution, however, have been constrained by detection limits and costs of the analytical methods and would be especially informed by time course studies of speciated chromium content. Much of what is considered generally known now about chromium kinetics has been inferred from studies comparing Cr(III) to Cr(VI) compounds, but could be refined by studies with speciated chromium measurements. Additionally, not all tissues have been routinely evaluated so that much of the understanding is also based on blood, urine and fecal excretion studies. Finally, there are limited studies comparing kinetics across species. Only two studies, Kargacin et al., 1993 and the NTP study (Stout et al., 2009) have evaluated both rats and mice. The NTP (2007) subchronic study also evaluated Guinea pigs.

3.2.1. In Vitro and Ex Vivo Studies of Bioavailability, Disposition, and Elimination

Wiegand et al. (1985) described the in vitro uptake kinetics of hexavalent chromium in erythrocytes of rats and humans. No large species differences were observed, with both species exhibiting Michaelis-Menten uptake kinetics, including an initial fast uptake rate (Table 3-3).

Table 3-3. In vitro kinetic parameters of hexavalent chromium uptake in RBCs of rats and humans

Hexavalent chromium uptake	Human	Rat
Half-time (whole blood)		
Initial phase	22.7 sec	6.9 sec
Second phase	10.4 min	10.1 min
Initial transport capacity (CrO_4^{2-}/erythrocyte/min)	3.1×10^8	2.5×10^8
Whole blood kinetics		
V_{max} (μmol/mL/min)	2.8	3.0
Michaelis constant (K_m) (mM/L blood)	20.9	14.1

Source: Wiegand et al. (1985).

$K_2Cr_2O_7$, a hexavalent chromium compound, introduced into plasma and reconstituted whole blood (stabilized with ethylenediaminetetraacetic acid [EDTA]) from three individuals was readily reduced to trivalent chromium in the concentration range of 100–1,000 µg hexavalent chromium/L (Corbett et al., 1998). Hexavalent chromium was detected in plasma when spiked at concentrations of 2,000 and 10,000 µg hexavalent chromium/L, but not at 1,000 µg hexavalent chromium/L. Furthermore, the plasma to erythrocyte ratio of total chromium decreased with increasing hexavalent chromium concentration. The variability between subjects in the ratio of plasma to erythrocyte total chromium diminished by approximately 1 order of magnitude as the hexavalent chromium concentration increased from 200 to 1,000 µg hexavalent chromium/L. Corbett et al. (1998) noted that these data suggest that the reductive capacity of erythrocytes is much greater than plasma, and that the reduction rate of hexavalent chromium in erythrocytes is greater than the rate of uptake from the plasma.

The partitioning of hexavalent chromium from plasma into erythrocytes is significant. It has been used as a biomonitoring endpoint (Kerger et al., 1996; Minoia and Cavelleri, 1988) and is responsible for the observed residence time of chromium in whole blood (Paustenbach et al., 1996; Langard et al., 1978).

3.2.2. In Vivo Studies of Bioavailability, Disposition, and Elimination

Most of the in vivo studies in both laboratory animals and humans that provide data on tissue uptake, disposition, and elimination of chromium evaluated total chromium and were limited to blood, urine and feces. Some studies did evaluate target tissues such as liver and kidney, and a few have evaluated uptake by the GI tract. As before, consideration of the analytical methods and the constraint on inferences to be drawn from total chromium measurements is an important consideration for evaluation of the data reliability and utility for risk assessment.

3.2.2.1. *Laboratory Animal Studies*

In rats gavaged with a single dose of $Na_2{}^{51}CrO_4$, approximately 99% of the administered dose was eliminated in the feces, while 0.8% was eliminated in the urine, both within 4 days (Sayato et al., 1980). Rats given 0.138 µmol/day hexavalent chromium (approximately 7 µg/day as $Na_2{}^{51}CrO_4$) for 3 days exhibited GI absorption of about 15% (Febel et al., 2001). Approximately 81 and 2.17% was eliminated in the feces and urine, respectively (Febel et al., 2001).

MacKenzie et al. (1959) performed several studies with radiolabeled chromium (delivered as $Na_2{}^{51}CrO_4$) to evaluate absorption determinants and tissue distribution. In the first study, a single oral gavage dose of 57 µg radiolabeled $Na_2{}^{51}CrO_4$ was administered to Sprague-Dawley rats. The chromium solutions were adjusted to pH 7.5 prior to administration. Half of

the animals were fasted prior to chromium administration, the other half were fed a stock diet ad libitum. Chromium concentrations in liver, kidney, stomach (plus contents), intestine (plus contents), blood, spleen, brain, lung, submaxillary gland, urine, and feces were analyzed at 1, 7, and 14 days post exposure. Table 3-4 summarizes the results; about 6% absorption occurred in the fasted animals and 3% absorption in the nonfasted animals. Significant initial distribution in the intestine was noted. The liver showed a maximal uptake of about 1% whereas the kidney, blood, and spleen had a maximal content of 0.1 to 0.2%. The splenic level persisted unchanged for 2 weeks post exposure. Most of the delivered dose was excreted in the feces.

Table 3-4. Distribution and retention of chromium in the rat after a single oral dose

	Percent of the administered dose/total tissue					
	Post exposure day 1		Post exposure day 7		Post exposure day 14	
Tissue	Fasted	Nonfasted	Fasted	Nonfasted	Fasted	Nonfasted
Stomach	1.964	2.22	0.02	<0.02	<0.02	<0.02
Intestine	26.78	18.2	0.07	0.04	0.05	0.03
Blood	0.17	0.03	0.05	0.03	0.05	0.03
Liver	1.03	0.21	0.14	0.03	0.07	0.03
Kidney	0.14	0.03	<0.02	0.02	—	—
Spleen	0.02	<0.02	0.02	<0.02	0.02	<0.02
Urine	2.9	0.63	5.3	2.3	5.7	2.8
Feces	64.1	73.5	90.2	95.3	94.7	96.4
Total, %	97.1	94.8	95.8	97.8	100.6	99.3

Source: MacKenzie et al. (1959).

In the second part of the same study, MacKenzie et al. (1959) administered 131 µg chromium (as $Na_2{}^{51}CrO_4$) and blood was removed from the heart at 4 hour post exposure for analysis of chromium in RBCs and plasma. In a third experiment, MacKenzie et al. (1959) also evaluated the role of the stomach wall in absorption. Using the same dosing, the influence of the stomach was bypassed by injecting chromium directly into the intestine, about 4 cm below the stomach. The RBC and plasma were measured for radioactivity 4 hour post exposure as in the second part of the study. Table 3-5 shows that the concentrations of chromium in whole blood, plasma, and RBCs were greater after administration to the intestine than to the stomach (0 indicates no significant count above background).

Table 3-5. Ratios (intestine:stomach) of chromium concentration in whole blood, plasma, and RBCs after a single oral dose

	Whole blood	Plasma	RBC
Cr(VI), fasted	1:3.94	1:2.10	1:6.03
Cr(VI), nonfasted	1:3.34	1:2.68	1:7.46
Cr(III), fasted	1:1.48	1:1.46	0:0
Cr(III), nonfasted	1:1.11	1:0.90	0:0

Source: MacKenzie et al. (1959).

Since RBC chromium is assumed to be in the hexavalent form and plasma chromium is assumed to be in the trivalent form, it appeared that there was some reduction of the hexavalent form in both fasted and nonfasted animals. Ratios for the counts in RBC to plasma (RBC:plasma) are shown in Table 3-6.

Table 3-6. Ratios of chromium concentration in RBCs and plasma in the stomach and intestine for fasted and nonfasted animals after a single oral dose

Organ and condition	RBC:plasma
Stomach, fasted	1:4.76
Stomach, nonfasted	1:8.8
Intestine, fasted	1:1.66
Intestine, nonfasted	1:3.17

Source: MacKenzie et al. (1959).

MacKenzie et al. (1958) administered potassium chromate (K_2CrO_4) in drinking water to Sprague-Dawely albino rats (n = 4/sex/group or n = 5/sex/control) at concentrations of 0, 0.45, 2.2, 4.5, 7.7 and 11 ppm (chromate ion) for one year. Two other groups were given water containing 25 ppm of either K_2CrO_4 or trivalent chromic chloride ($CrCl_3$) for the same period. Chromium was analyzed in liver, kidneys, and femurs at 6 months and in these tissues plus spleen after one year by Saltzman's diphenylcarbazide method of permanganate oxidation. No changes in weight gain or food consumption were reported, but water intake was decreased to 77% in females and 84% in males. Resultant tissue concentrations for the rats receiving K_2CrO_4 are presented in Table 3-7. The order of chromium concentrations in tissues was as follows: spleen > bone > kidney > liver. No gender-specific differences in chromium tissue accumulation were observed. An appreciable increase in all tissues examined occurred when the animals received between 5 and 10 ppm K_2CrO_4. Concentrations in these tissues were approximately

ninefold higher in the group given K_2CrO_4 than those receiving the trivalent compound, again suggesting the greater bioavailability of hexavalent chromium.

Table 3-7. Terminal tissue chromium levels in rats ingesting potassium chromate (K_2CrO_4) in drinking water for 1 year

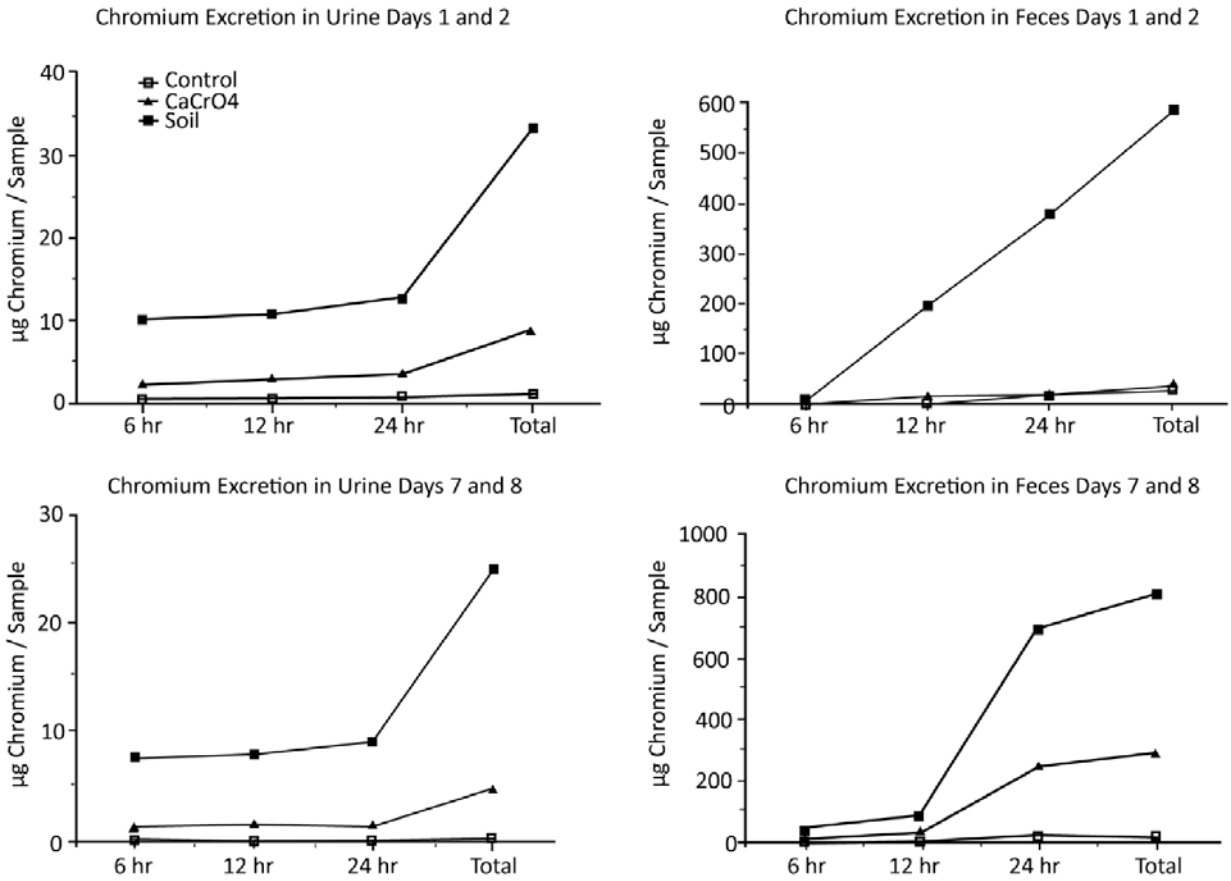
$K_2Cr_2O_4$ concentration (mg/L)	Liver (µg/g)		Kidney (µg/g)		Bone (µg/g)		Spleen (µg/g)	
	Male	Female	Male	Female	Male	Female	Male	Female
Controls	0	0	0	0.25 ± 0.02	0	0.72 ± 0.8	0	0
0.45	0.02 ± 0.002	0.08 ± 0.007	0.14 ± 0.007	0.39 ± 0.04	0.58 ± 0.04	0.76 ± 0.04	0.95	0.91 ± 0.11
2.2	0.08 ± 0.017	0.17 ± 0.03	0.29 ± 0.02	0.48 ± 0.07	1.27 ± 0.06	1.48 ± 0.04	0.68 ± 0.18	1.14 ± 0.1
4.5	0.15 ± 0.04	0.47 ± 0.06	0.45 ± 0.17	1.09 ± 0.13	2.14 ± 0.25	2.44 ± 0.25	3.41 ± 0.44	4.48 ± 0.71
7.7	0.70 ± 0.04	0.55 ± 0.06	3.30 ± 0.03	2.39 ± 0.09	3.43 ± 0.83	5.10 ± 0.35	5.24 ± 0.20	4.73 ± 0.8
11.2	1.22 ± 0.06	1.62 ± 0.14	4.40 ± 0.36	3.98 ± 0.32	3.84 ± 0.49	6.06 ± 0.58	9.91 ± 0.83	11.1 ± 0.86

Source: MacKenzie et al. (1958).

Coogan et al. (1991a) exposed male F344 rats to hexavalent chromium as potassium chromate (K_2CrO_4) dissolved in their drinking water at concentrations of 100 and 200 ppm for 3 or 6 weeks. Total chromium concentrations were measured in lung, liver, kidney, and blood by AAS after acid digestion. Drinking water consumption was reduced at both concentrations for the first 3 weeks and then only at the higher concentration the second 3 weeks. Chromium was not detected in any lung samples. At both concentrations and durations, the order of tissue chromium concentrations was as follows: kidney > liver > blood. Although a general trend of increasing chromium content as a function of exposure duration existed for the liver, kidney, and blood samples analyzed, only the kidney samples were significantly different between the 3- and 6-week sacrifices ($p < 0.05$). Blood chromium levels were not significantly different at either sacrifice or at either concentration.

Witmer et al. (1991, 1989) performed several studies to evaluate the toxicity of chromium contaminated soil samples from sites in Jersey City, NJ. In all studies, the organs evaluated for chromium content included the liver, lung, spleen, kidney, muscle, brain, and testes. An aliquot of blood from the abdominal aorta was also analyzed. Chromium was identified in samples by the Baird ICP method (urine and feces) or by atomic absorption using a graphite furnace (other tissues). In the initial pilot study, male Sprague Dawley rats were dosed by gavage with 0, 20, 40 and 100 µmole/kg hexavalent chromium as $Na_2CrO_4 \cdot 4H_2O$ in distilled water for 7 days. Recovery of chromium was low, tissue burdens at the highest dose represented only 1.7% of the amount administered. When tissue burden was expressed on a µg/g basis, the kidney contained the highest amount with liver and blood also containing greater amounts than the other samples. In the next experiment, doses of 120 µmole Cr/kg were administered to Sprague-Dawley rats (n = 3/group) using four sources of chromium: 1) Na_2CrO_4, 2) $CaCrO_4$, 3)

Pacific Avenue Fines [PAC] soil sample obtained from the NJDEP, and 4) a mixture of soil and calcium chromate containing 60 µmole Cr/kg each. Recovery was again low whether expressed as percent of total administered dose or based on the last dose before sacrifice, with the sampled tissues accounting for less than 2% of the total administered dose, suggesting minimal tissue storage; whatever the source, the kidney had the highest tissue concentrations. The absorption from the soluble sodium salt was generally higher in all tissues studied than from the calcium salt, soil, or mixture. In a third study, groups of male Sprague Dawley rats (n = 6/group) were orally gavaged with chromium in corn oil and at a higher concentration (240 µmol Cr/kg) from the same four sources for a longer duration (14 days). The total tissue levels again represented a small percentage of the chromium administered, but this time, blood levels were higher than those found in the kidney. Thus, to determine if the major portion of the orally administered chromium was rapidly excreted, urine and feces were collected in the last experiment in which rats (n = 3/group) were gavaged with 240 µmol Cr/kg as the calcium salt or in contaminated soil. Dosing was carried out once daily at the same time each day for eight days. Urine and feces were collected at 6, 12, and 24 hour after dosing on days 1 and 2 and on days 7 and 8; chromium in the samples was measured by the ICP method. Results for urine and feces on the different days are shown in Figure 3-2. The data indicate that chromium is not excreted under these conditions of corn oil dosing to any appreciable extent in the urine, but some significant excretion occurs in the feces. The patterns also show that more of the chromium from soil was excreted at both sample periods in the urine and feces than from the rats treated with $CaCrO_4$.



Source: Witmer et al. (1991, 1989).

Figure 3-2. Chromium excretion in urine and feces of Sprague-Dawley rats. Rats were gavaged with corn oil (control), calcium chromate, or chromium-contaminated soil at a level of 240 µmole Cr/kg daily for either 2 days (upper two panels) or 8 days (lower two panels). Urine and feces were collected at 6, 12, and 24 hour after each dosing period. Chromium levels shown are for each time period as well as for the total 24-hour period.

As part of the development of the physiologically-based pharmacokinetic (PBPK) model discussed in Section 3.5, O'Flaherty and Radike (1991) dosed male Sprague-Dawley rats with either trivalent chromic chloride-hexahydrate ($CrCl_3 \cdot 6H_2O$) or hexavalent sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) administered either in drinking water or by inhalation. The inhalation concentration (for 6 hour/day) chosen (200 µg Cr/m^3) was based on the lack of toxicity observed at that same concentration in a study conducted in rats by Glaser et al. (1985). The control group was exposed to filtered air for the inhalation study and received deionized water for the ingestion study. Chromium aerosols were generated from solutions by ultrasonic jet nebulizers and chromium concentrations were measured daily by AAS; specific Cr(VI) analysis was performed weekly by the diphenylcarbazide method (NIOSH method 7600). The mass mean aerodynamic diameter (MMAD) for the Cr(III) aerosol was 0.9 µm ± 0.28, 80% respirable; and the Cr(VI) MMAD was 1.0 µm ± 0.24, 63% respirable. The concentration in drinking water (deionized) of 12.9 mg Cr/L was based on the dose calculated to be delivered by the inhalation route in a 200 g rat. Each exposure group contained 36 rats; 6 rats from each group were sacrificed following days 2, 5, 10, 20, and 40 of exposure. The 36 rats remaining after the 40-day exposure were allowed to live untreated 20 days longer (day 60 of experiment) to ascertain if there were clearance differences among treatment groups. Blood, urine, and fecal chromium levels were monitored on days 2, 5, 10, 20, and 40 for the 40-day exposure period, and again at 20 days post exposure. Chromium content in blood and urine were measured by ICP-MS. Tissues were digested in acid, ashed, and chromium content determined by AAS. Tissue total chromium content was determined in kidney, liver, muscle, intestine, lung, and carcass. The summary of the experimental data for the ingestion route is provided in Table 3-8.

Table 3-8. Time course of chromium tissue concentrations in male Sprague-Dawley rats[1] ingesting 12.9 mg Cr/L of trivalent chromic chloride-hexahydrate or hexavalent sodium dichromate in drinking water for 40 days

Day of study	Lung µg Cr/g	Liver µg Cr/g	Intestine µg Cr/g	Kidney µg Cr/g	Muscle µg Cr/g	Blood ng Cr/ml	Urine µg Cr/day	Feces mg Cr/day
Control								
2	ND[2]	ND	0.65	1.58	Trace	1.5	0.017	ND
5	ND	ND	0.83	ND	Trace	1.6	ND	0.002
10	ND	ND	0.56	ND	ND	4.2	0.003	ND
20	ND	ND	0.85	ND	Trace	3.4	ND	0.013
40	ND	0.035	0.68	ND	Trace	6.8	0.010	ND
60	ND	0.032	0.72	ND	0.038	2.5	ND	ND
Trivalent chromic chloride hexahydrate								
2	ND	0.042	18.3	ND	ND	2.48	0.227	0.821
5	ND	Trace	17.2	ND	ND	3.11	0.065	0.729
10	ND	0.034	20.6	ND	ND	16.8	0.040	1.20
20	ND	ND	26.8	ND	ND	5.60	0.075	1.07
40	ND	ND	7.15	ND	ND	4.72	0.017	1.12
60	ND	Trace	0.83	ND	ND	5.52	2.01	ND
Hexavalent sodium dichromate								
2	ND	0.209	15.5	0.249	ND	9.0	0.622	0.997
5	ND	0.372	22.7	0.588	ND	11.8	1.79	0.835
10	ND	0.585	14.4	1.60	ND	18.5	2.01	0.949
20	1.17	1.18	29.0	1.71	0.077	48.9	3.08	0.977
40	0.65	1.50	6.8	1.909	0.103	58.3	2.19	1.51
60	0.65	0.509	0.83	0.634	0.070	11.3	0.217	ND

[1]n = 6 per time point per exposure group
[2]Non detect

Source: O'Flaherty and Radike (1991).

Kargacin et al. (1993) examined the species differences in distribution of total chromium in male C57BI/6J mice and F344 rats exposed to 130 ppm potassium chromate (K_2CrO_7) in drinking water (8 mg hexavalent chromiujm/kg-day) for 4 or 8 weeks. Total concentrations in the tissues listed in Table 3-9 were measured by AAS after acid digestion. Regardless of duration, chromium accumulated primarily in the spleen, liver, kidney, and bone of mice and rats, with mouse liver, spleen, and bone burdens being, on average, several-fold higher than in rats (Table 3-9). The reason for the higher accumulation of chromium in mouse liver is unknown, but may result from greater reduction of hexavalent to trivalent chromium in the rat gut limiting uptake of chromium from the GI tract. Alternatively, the mouse liver may have a higher hexavalent chromium reduction capacity than rats, causing more reduced trivalent chromium to be sequestered in hepatocytes.

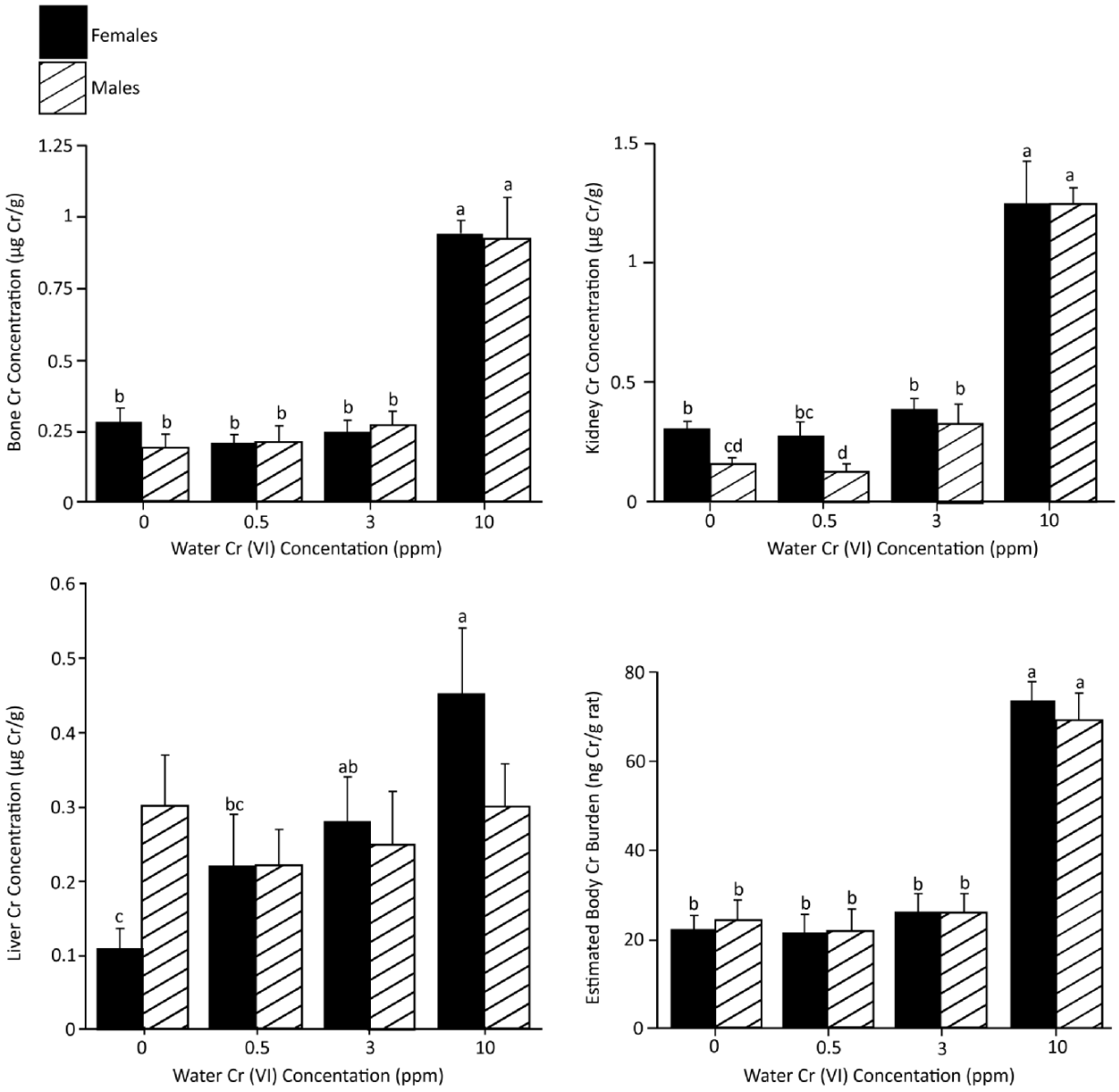
Table 3-9. Chromium in tissues (µg/g wet tissue or µg/mL blood) of mice and rats after ingesting K_2CrO_7 in drinking water (8 mg hexavalent chromium/kg-day) for 4 or 8 weeks

	Controls	4-Week exposure	8-Week exposure
Mice			
Liver	0.22 ± 0.14	10.92 ± 5.48	13.83 ± 6.06
Kidney	0.24 ± 0.14	3.77 ± 0.99	4.72 ± 0.68
Spleen	0.53 ± 0.38	5.04 ± 1.45	10.09 ± 2.50
Femur	0.90 ± 0.48	7.43 ± 1.03	12.55 ± 2.99
Lung	0.24 ± 0.12	0.99 ± 0.10	1.08 ± 0.26
Heart	0.32 ± 0.15	0.80 ± 0.23	1.02 ± 0.20
Muscle	0.32 ± 0.23	1.12 ± 0.37	0.60 ± 0.25
Blood	0.14 ± 0.05	0.71 ± 0.07	0.42 ± 0.04
Rats			
Liver	0.19 ± 0.14	3.32 ± 0.93	3.59 ± 0.73
Kidney	0.34 ± 0.20	8.62 ± 2.40	9.49 ± 4.38
Spleen	0.43 ± 0.20	3.65 ± 1.87	4.38 ± 0.84
Femur	1.00 ± 0.46	1.85 ± 0.46	1.78 ± 0.99
Lung	0.39 ± 0.43	1.10 ± 0.38	0.67 ± 0.24
Heart	0.38 ± 0.22	0.52 ± 0.12	1.05 ± 0.19
Muscle	0.24 ± 0.14	0.19 ± 0.10	0.17 ± 0.10
Blood	0.19 ± 0.17	0.73 ± 015	0.58 ± 0.13

Source: Kargacin et al. (1993).

Sutherland et al. (2000) determined tissue concentrations of chromium in male and female F344 rats (n = 7/sex/group) that drank hexavalent potassium chromate (K_2CrO_4) ad libitum at 0, 0.5, 3.0 and 10.0 ppm for 44 weeks. Solutions were prepared in deionized water weekly; consumption rates were recorded weekly. Rats were switched to deionized water only 4

to 6 days prior to sacrifice to ensure tissue concentrations did not reflect recent exposure. Rats were euthanized by i.p. injection of sodium pentobarbital and exsanguinated prior to tissue harvest. Kidneys (0.6–1.4 g) were digested in 1 N HNO_3 and chromium content measured by atomic absorption spectrophotometry with deuterium background correction. Liver (0.7–1.2 g), brain (0.8–1.1 g), bone (0.5–1.0 g), whole blood (1.5–2 ml), testes (0.7–1.3 g), and ovarian (0.1–0.2 g) samples were digested in a low trace metal reagent grade $HNO_3/HCLO_4$ mixture and analyzed by inductively coupled plasma (optical) – atomic emission spectrophotometry (ICP-AES) or inductively coupled plasma – mass spectrophotometry (ICP-MS). Because bone samples were optically saturated with calcium and phosphorous, they were reanalyzed with ICP-MS. ICP-MS was also used for the testis and ovarian samples due to their small size and the better detection limit for this method. Detection limits of the ICP-AES and ICP-MS methods were 5 and 2.5 ppb chromium in solution. Analytical accuracy was assessed by measuring total chromium in liver, bone, brain, and testis samples spiked with a known amount of Cr(VI). Recovery of chromium in these spiked samples was 101.7 ± 2.9% (range = 92.3–116.4%). Additionally, spikes of chromium from a National Institute of Standards and Technology (NIST) source independent of instrument calibration standards were also analyzed as unknowns and average recovery was 95.7 ± 1.3% (range 86.5–100.6%). Tissue (bone, kidney, liver) and total body burdens are shown in Figure 3-3. Chromium was most concentrated in kidney and bone in this study, results consistent with those of MacKenzie et al. (1966) and Witmer et al. (1985). Despite consuming more water than the males, the females did not have significantly higher body burdens (Panel D, Figure 3-3) than males. The lack of difference between the sexes may be due to the contribution of testicular burdens to the total, or perhaps the chromium was either less bioavailable in females or cleared more effectively. Significant tissue accumulation occurred at the 3 and 10 ppm exposure levels, with the effect most pronounced at 10 ppm, indicating that a portion of the Cr(VI) escaped extracellular reduction in the GI tract and became bioavailable for systemic distribution. An alternate mechanism proposed for the findings was that Cr(III) formed in the GI tissues and absorbed was not cleared in the kidneys and was taken up by the cells. Male rats showed a greater concentration in kidneys at the 3 ppm level than did the females, of note since the females consumed more Cr per gram body weight. The liver was the only tissue in female rats in which significantly elevated concentrations of chromium could be found after ingestion of Cr(VI) at the 3 ppm level. Testicular concentrations were slightly elevated in rats that drank 10 ppm Cr(VI). Brain, ovarian, and whole-blood concentrations were below detection limits in all exposed groups. The lack of concentrations in whole-blood was attributed to rapid delivery of Cr to tissues and clearance of plasma Cr.



Source: Sutherland et al. (2000).

Figure 3-3. Chromium concentrations in male and female F344 rats following chronic drinking water consumption of Cr(VI). Bone (upper left), renal (upper right), and liver (lower left) tissue burdens or total body burden (lower right) are mean ± SE. Means that do not share a common superscript are significantly different ($p \leq 0.05$).

The NTP conducted a comparative absorption subchronic study in rats, mice, and guinea pigs prior to conducting the 2-year bioassay (NTP, 2007; Appendix G). Guinea pigs were chosen for the study because they more closely resemble humans in that they do not have a forestomach and require a reducing agent (Vitamin C) in their diet. Sodium dichromate dihydrate was administered ad libitum in drinking water to male F344/N rats, $B6C3F_1$ mice, and Hartley guinea pigs (n = 4/species/dose) at dose concentrations of 0, 2.87, 8.62, 28.7, 86.2, 287, and 862 mg Cr(VI)/L (equivalent to 0, 1, 3, 10, 30, 100, and 300 mg Cr/L) for 21 days, followed by 2 days of drinking water alone. Animals were sacrificed on day 24, and total chromium concentrations in blood, kidney, and femur (rats only) were determined. Blood and renal tissue concentrations increased in all three species with dose. A statistically significant and apparently nonlinear uptake in blood and kidney at the two highest concentrations was evident in all species and in guinea pigs at 30 ppm. Uptake in guinea pigs did not appear to generally differ from that of rodents, suggesting that the lack of a forestomach did not alter GI uptake appreciably. Values for the rats and mice were in agreement with those previously published by Sutherland et al. (2000).

As part of the 2-year NTP cancer bioassay (NTP; Stout et al., 2009) discussed in Section 4, a total chromium tissue distribution study was also conducted (NTP, 2008; Appendix J). Groups of 40 male F344 /N rats and female $B6C3F_1$ mice were randomly assigned to the tissue distribution study at the beginning of the 2-year bioassay and treated identically to the core study groups. Animals were exposed to sodium dichromate dehydrate ($Na_2Cr_2O_7 \cdot 2H_2O$) in drinking water at concentrations of 0, 14.3, 57.3, 172, or 257.4 mg/L for 53 weeks. Equivalent Cr(VI) concentrations based on the percent mass of Cr in sodium dichromate dehydrate are 0, 5, 20, 60 and 190 mg/L. Dose formulations were prepared approximately every two weeks in tap water. On days 4, 11, 180, and 369, up to 10 animals/dose group were removed from treatment and placed in individual metabolism cages to allow for separate collection of urine and feces for 48 hours. Two collections of urine and feces were made to include the intervals from 0 to 24 and 24 to 48 hour; measured values were combined to yield the reported 48-hour values. At the end of 48 hours, animals were anesthetized with CO_2/O_2 for retroorbital sinus sampling of blood. Blood was separated into cells and plasma. While the animals remained anesthetized the aorta was severed and the abdominal wall opened to obtain liver, kidneys, and stomach (separated into glandular and forestomach). Only plastic, ceramic, Teflon, or tungsten carbide instruments were used to avoid chromium contamination from stainless steel. Tissue samples were digested in concentrated nitric acid. Chromium content of the experimental samples was analyzed by ICP-MS using spiked internal standards; with calibration performed prior to each analysis. Fecal and urinary chromium concentrations expressed as µg were significantly elevated relative to controls at all concentrations and at all sacrifice times in both the male rats and female mice, with the majority of the chromium in the feces. Plasma and RBC chromium concentrations (µg/g) were

consistently significantly elevated relative to controls at the two highest exposure levels. Tissue chromium concentrations were significantly elevated relative to control at all concentrations (Shirley's test; $p < 0.05$ at lowest exposure level and $p < 0.01$ at all others) in both liver and kidney samples, with liver concentrations higher than kidney values in both the rats and mice. The tissue chromium concentrations for the glandular and forestomach samples in both species are presented in Table 3-10. As for the other tissues sampled, chromium concentration in the glandular stomach and forestomach were significantly elevated relative to controls at the two highest concentrations. Stout et al. (2009) calculated the average daily dose in mg/kg for animals on test in the main study using body weight and water consumption data as follows:

Sodium dichromate dihydrate (mg/L):	**0**	**14.3**	**57.3**	**172**	**516**
Average daily dose (mg/kg):					
Male rats	0	0.6	2.2	6	17
Female mice	0	1.1	3.9	9	25
Ratio (mice:rats)	0	1:1.83	1:1.77	1:1.50	1:1.47

Assuming a similar consumption and weight pattern in these satellite animals, it can be seen that the mice generally consumed 1.5 to 1.8 more chromium as an average daily dose (mg/kg), which may account to some degree for the larger tissue concentrations in this species observed in Table 3-10.

Table 3-10. Tissue concentrations of chromium in male F344/N rats and female B6C3F$_1$ mice in the 2-year NTP drinking water study of sodium dichromate dihydrate

Tissue (µg/g)[1]	0 mg/L	14.3 mg/L	57.3 mg/L	172 mg/L	516 mg/L
	Rats				
Glandular stomach					
Day 6	0.076 ± 0.003	0.143 ± 0.008*	0.333 ± 0.040**	0.773 ± 0.105**	1.967 ± 0.109**
Day 13	0.095 ± 0.008	0.254 ± 0.073*	0.310 ± 0.049*	1.331 ± 0.060**	1.762 ± 0.042**
Day 182	0.197 ± 0.031	0.414 ± 0.012*	1.043 ± 0.081**	4.300 ± 0.367**	9.886 ± 0.354**
Day 371	0.253 ± 0.066	0.334 ± 0.029	1.038 ± 0.115*	4.801 ± 0.345**	14.643 ± 0.121**
Forestomach					
Day 6	0.098 ± 0.024	0.076 ± 0.004	0.122 ± 0.008	0.294 ± 0.029	0.285 ± 0.283
Day 13	0.091 ± 0.015	0.102 ± 0.034	0.171 ± 0.050	0.221 ± 0.055*	0.593 ± 0.159**
Day 182	0.089 ± 0.018	0.099 ± 0.003	0.338 ± 0.022*	0.574 ± 0.171*	1.654 ± 0.244**
Day 371	0.090 ± 0.015	0.118 ± 0.008	0.328 ± 0.081*	1.338 ± 0.444**	2.849 ± 0.975**
	Mice				
Glandular stomach					
Day 6	0.306 ± 0.056	0.645 ± 0.253	1.258 ± 0.290*	2.450 ± 0.266**	5.785 ± 0.131**
Day 13	0.207 ± 0.053	0.324 ± 0.030	2.614 ± 0.190*	7.048 ± 1.751**	13.130 ± 2.604**
Day 182	0.305 ± 0.078	0.644 ± 0.035*	3.659 ± 0.547**	11.520 ± 3.017**	52.673 ± 12.310**
Day 371	0.731 ± 0.306	0.676 ± 0.104	2.807 ± 0.330*	9.994 ± 1.079*	49.867 ± 12.251**
Forestomach					
Day 6	0.328 ± 0.132	0.683 ± 0.262	1.308 ± 0.553	1.102 ± 0.373	1.286 ± 0.116
Day 13	0.201 ± 0.094	0.288 ± 0.056	0.400 ± 0.044	2.030 ± 0.532*	3.849 ± 1.811*
Day 182	0.173 ± 0.064	0.444 ± 0.099	1.033 ± 0.102*	2.141 ± 0.643**	9.624 ± 3.638**
Day 371	0.320 ± 0.049	0.381 ± 0.077	1.271 ± 0.300*	1.812 ± 0.208*	7.442 ± 0.764**

[1]n = 3; mean ± SE. Statistical tests performed on unrounded data.
*significantly different from the control group ($p \leq 0.05$) and ** ($p \leq 0.01$) by Shirley's test

Source: NTP (2008; Appendix J).

Hexavalent chromium is capable of crossing the placenta. Pregnant mice given a single intravenous injection of 10 mg hexavalent chromium/kg (as $Na_2{}^{51}CrO_4$) on gestation day (GD) 13 exhibited total embryo chromium levels that were 12% of maternal blood levels (Danielsson et al., 1982). Intraperitoneal injection of 10 mg trivalent chromium/kg (as $^{51}CrCl_3$) in pregnant mice on GD 8 resulted in approximately equal [^{51}Cr] activity in the embryo and maternal blood (Iijima et al., 1983). While these studies demonstrate placental transfer of chromium, they are of limited use for assessing embryonic exposure to chromium as a result of maternal oral exposures to hexavalent chromium.

3.2.2.2. *Human Studies*

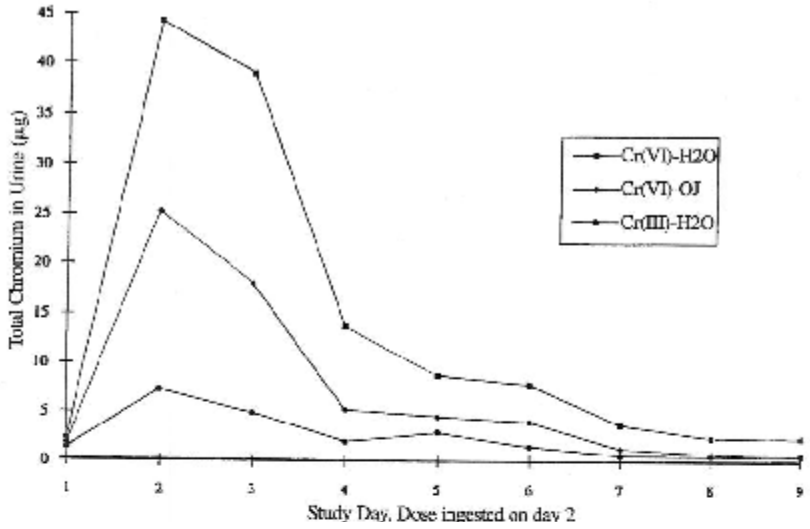
Most quantitative studies of the GI absorption of both hexavalent and trivalent chromium in humans have estimated the absorption fraction to be <10% of the ingested dose. In general, these studies suggest that the absorbed fraction of soluble hexavalent chromium compounds (e.g., $K_2Cr_2O_7$) is higher than insoluble forms; and soluble hexavalent chromium compounds are absorbed to a greater extent than soluble trivalent chromium compounds (e.g., $CrCl_3$).

Absorption and elimination of trivalent and hexavalent chromium, following ingestion by human volunteers of either single or multiple drinking water doses, were evaluated in a series of studies (Finley et al., 1996, 1997; Kerger et al., 1996, 1997; Paustenbach et al., 1996). Collectively, these studies illustrate absorption and elimination kinetics for human volunteers that provide critical data for the interpretation of biomonitoring in blood and urine, but the disposition of chromium and in particular its speciation in different tissue compartments, can only be inferred by comparison of these same rates (i.e., absorption and elimination) between chromium (III) and chromium (VI) compounds. Nevertheless, they can provide a basis for human model development (see Section 3.5) and a comparison for the laboratory animal studies. Considerable variability across the human volunteers was noted in these studies, and may reflect interindividual differences that influence gastric reduction such as time since last meal or volume of the contaminated water ingested, but may also reflect different genetic capacities for Cr(VI) reduction.

Of note, a human use committee reviewed the protocols prior to initiation and concluded that the study design was adequate to meet the objectives of the study; that the dosing would not pose a health risk to the participants, and that the participants were properly informed of potential risks.

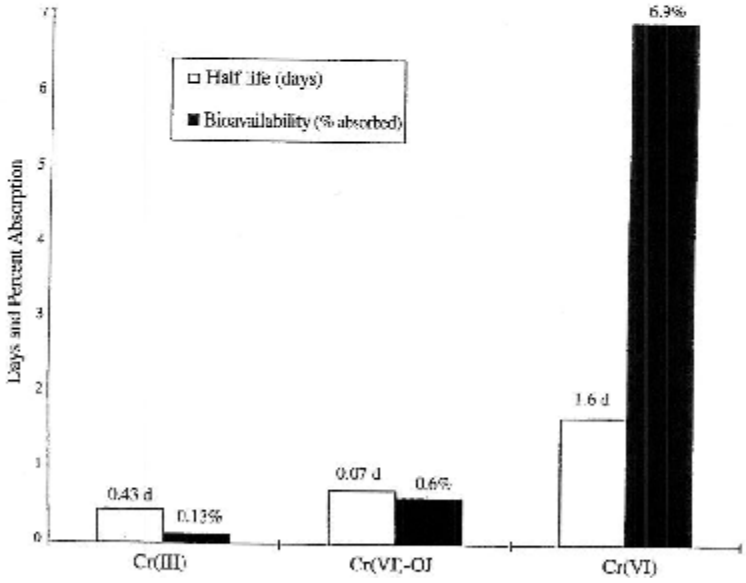
Kerger et al. (1996) examined the absorption (first 2 hours) and elimination (up to 14 days) kinetics of three different chromium compounds: 1) chromic chloride (Cr(III) as $CrCl_3$); 2) potassium dichromate reduced with orange juice (Cr(VI)-OJ); and 3) potassium dichromate (Cr(VI) as $K_2Cr_2O_7$). In each experiment, three or four adult male volunteers ingested a single bolus dose of 5 mg Cr (0.5 L of 10 ppm chromium solution in deionized water). One volunteer was common to each of the experiments. Their diet was not controlled, but participants were

prohibited from ingesting vitamin supplements containing Vitamin C or chromium. A detailed log of all intakes (food, beverage, and dietary supplementation) was maintained throughout the duration of the study. Urine voids were collected beginning with the 1st morning void through the last day of the study. Blood samples were drawn, centrifuged and pooled for analysis of RBC and plasma at various intervals. All urine samples were analyzed for total chromium, total urine volume, specific gravity and creatinine. Total chromium was measured in urine, plasma and RBC samples by graphite furnace AAS according to EPA Method 218.2 as modified by Pautenbach et al. (1996); the limit of detection was 0.5 µg Cr/L of urine, 0.3–0.5 µg Cr/L of plasma, and 0.2 µg Cr/L of blood. The absorption was highly dependent on the form of chromium ingested. $CrCl_3$ was poorly absorbed (estimated 0.13% bioavailability) and rapidly eliminated in urine (excretion half-life, $T_{1/2}$, ~10 hour), whereas Cr(VI)-OJ was absorbed more efficiently (0.60% bioavailability) and eliminated more slowly ($T_{1/2}$ ~17 hour). Cr(VI) had the highest bioavailability (6.9%) and longest $T_{1/2}$ (~39 hour). All three compounds caused temporary elevation in RBC and chromium concentrations with the magnitude and duration of elevation exhibiting a clear trend: Cr(VI) > Cr(VI)-OJ > Cr(III). Figure 3-4 shows a comparison of the absorption/elimination profiles for the three chromium compounds. Figure 3-5 shows a comparison of the percent bioavailability and elimination $T_{1/2}$. Plasma chromium concentrations peaked within 90 minutes after the Cr(III) dosing and averaged 2.8 µg Cr/L (range 1.3–3.7). Potassium dichromate reduced in orange juice resulted in a lesser elevation, with an average of 2.2 µg Cr/L; whereas potassium dichromate resulted in an average peak concentratin of 26 µg Cr/L (range 5.1–57). The peak chromium concentrations in RBC mirrored those in plasma, with the peak average concentrations for the Cr(III), Cr(VI)-OJ and Cr(VI) reported as 7.5 (range 5.1–14), 5.5 (range 5.1–6.1), and 17.6 (range 13.5–24) µg Cr/L, respectively. Because the Cr(VI) increases in RBC provide a specific indication of chromium in the hexavalent state, these data suggest that at the low doses tested, there was predominant reduction of the ingested Cr(VI) in the stomach and small intesting followed by systemic uptake. Distribution and excretion was postulated to be as Cr(III) organic complexes. Because the increases in chromium content were always similar for plasma and RBC, an equilibrium between the cellular and noncellular compartments of the blood was suggested and considered to be consistent with the kinetic behavior of absorbed Cr(III). The higher bioavailability of the Cr(VI)-OJ compound could represent the formation of highly soluble chromium complexes (e.g., ligands with acorbate or sulfhydryl proteins) that were rapidly cleared from plasma via tissue uptake or kidney filtration. Alternatively, it is possible that a small fraction was absorbed as Cr(VI) where it encountered additional reducing agents and potential complexation ligands that produced a variety of chromium complexes with different kinetic patterns. Although it is unlikely that the reducing capacity of the blood was overwhelmed at the absorbed doses in this study, elucidation of the mechanisms would require additional investigations and be greatly informed by analytical techniques capable of differentiating the speciated state in biological samples.



Source: Paustenbach et al. (1997).

Figure 3-4. Chromium absorption and elimination in human volunteers after ingestion of a single bolus dose in drinking water.



Source: Paustenbach et al. (1997).

Figure 3-5. Biovailability and elimination half-life for chromium ingested by human volunteers as a single bolus dose in drinking water.

Kerger et al. (1997) next explored the hexavalent chromium absorption, distribution, and excretion following oral exposure in human volunteers (n = 5 adults) to 5 or 10 mg Cr(VI)/L in drinking water administered as either a single bolus dose (0.5 L swallowed in 2 minutes) for a total dose of 5 or 10 mg; or for 3 days at a dosage of 1 L/day (3 doses of 0.33 L each day, at 6-hour intervals) for a total dose of 15 or 30 mg. The source of Cr(VI) was potassium dichromate ($K_2Cr_2O_7$) for the single dose experiment and potassium chromate (K_2CrO_4) for the multiple-dose experiment. Other experimental methods and total chromium analyses in the samples were as in Kerger et al. (1996); a study of the stability of the dosing solution confirmed no measurable reduction of Cr(VI) occurred throughout the study. The percent uptake of total chromium measured in the urine was 5.7% for the bolus dose at 10 mg Cr(VI)/L, and 1.7 and 3.4% for the multiple doses at 5 and 10 mg Cr(VI)/L, respectively. Plasma and RBC total chromium levels were consistent in timing and magnitude, with a temporary elevation about 60 minutes after bolus dosing. The average peak concentration of total chromium after the multiple dosing regimen in plasma and RBC showed dose trends and timing generally consistent with the bolus dose experiment, but with greater interindividual variability. Urine and plasma samples taken during day 1 of the single bolus experiment were also assayed for Cr(VI) according to EPA method 218.6, using ion chromatographic separation with post-column color development and spectroscopic detection, with a detection limit of 1–2 µg Cr(VI)/L. All samples were found to contain no traces of Cr(VI) at any time point, including during the rapid uptake phase. These observations are consistent with the interpretations of Kerger et al. (1996) regarding the reduction, uptake, distribution and elimination of Cr(VI) – that Cr(VI) is reduced to Cr(III) with multiple doses at 5 and 10 mg Cr(VI)/L. Additionally, the data were considered consistent with published kinetic models of Cr(III) behavior in animals (Aitio et al., 1988; Lim et al., 1983). Aitio et al. (1988) developed a compartmental model of the half-life of Cr(III) in humans that was based on distribution and elimination rates from three separate compartments: the fast-elimation ($T_{1/2}$ = 7 hour), the moderate elimination ($T_{1/2}$ = 15 days), and slow elimination ($T_{1/2}$ = 3 years) compartments. Lim et al. (1983) described a similar compartmental model, suggesting Cr(III) half-lives of 0.5–12 hour in blood (fast compartment), 1–14 days in storage organs such as the liver and spleen (medium compartment), and 3 – 12 months in other solid tissues (slow compartment).

Finley et al. (1996) evaluated urinary chromium clearance in six healthy, adult (ages 25–39 years; four males weighing 79–97 kg, two females weighing 56–62 kg) human volunteers. The entire chromium ingestion and urine collection period covered 18 days and was divided into five separate, but consecutive, phases as described below:

Days 1 – 7:	Chromium picolinate ingestion (loading dose)
Days 8 – 10:	Cr(VI) ingestion

Days 11 – 13:	No-dose period
Days 14 – 16:	Cr(III) ingestion
Days 17 – 18:	Post-dose period

The dosing regimen was designed to achieve a steady-state urine concentration during dosing and to ensure a return to baseline between dosing with Cr(VI) and Cr(III). Chromium picolinate was delivered at a dose of 0.2 mg for the first seven days, considered a loading dose for this dietary supplement. Cr(VI) was delivered as potassium dichromate ($K_2Cr_2O_4$) on days 8 through 10 in a capsule at a dose of 0.005 mg Cr(VI), the U.S. EPA RfD for Cr(VI) at that time. Cr(III) was then ingested as chromic oxide (Cr_2O_3) at 1.0 mg Cr(III)/kg/day, the U.S. EPA RfD level for Cr(III) at that time, on days 14 through 16. Urine samples and measurements were performed as previously described for Kerger et al. (1996, 1997). The ingestion of chromium picolinate resulted in significantly elevated urine concentrations such that participants routinely exceeded background. Ingestion of Cr(VI) yielded individual mean total urinary chromium levels of 1.2–23 µg/L, and a pooled mean value of 2.4 µg/L. Ingestion of the Cr(III) compound yielded no significant increases in urinary chromium concentrations, suggesting negligible absorption.

Paustenbach et al. (1996) evaluated uptake and elimination in a male, Caucasian volunteer (age 44 years) who ingested deionized water containing potassium dichromate ($K_2Cr_2O_4$) in five daily portions (400 mL, 2 mg Cr(VI)/L each) for 17 days. Methods described for the Kerger et al. (1996) study were used to sample and quantify chromium in blood and urine. Bioavailability was estimated at 2% and the plasma elimination half-life at 36 hour, both consistent with the previous studies. The time to achieve steady-state concentrations in urine and blood was 7 days. Both plasma and RBC chromium concentrations returned rapidly to background levels within a few days, again suggesting that concentrations of 10 mg Cr(VI)/L or less in drinking water of humans appears to be completely reduced to Cr(III) prior to systemic distribution.

Finley et al. (1997) extended this work by evaluating chromium kinetics in human volunteers following repeated oral exposure to Cr(VI) concentrations ranging from 0.1 to 10.0 mg/L. Five healthy, adult (age 30 to 54 years), male Caucasian volunteers ingested a liter (in three volumes of 333 ml at approximate 6-hour intervals) of deionized water containing Cr(VI) concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 mg Cr(VI)/L. Potassium chromate (K_2CrO_4) was used as the source of soluble Cr(VI). Other experimental methods and total chrome analyses in the samples were as in Kerger et al. (1996); a study of the stability of the dosing solution confirmed no measurable reduction of Cr(VI) occurred throughout the study. Each of the five subjects demonstrated an increase in the amount of urinary chromium excreted, ranging from a mean of 1.7% at the low dose (0.1 mg Cr(VI)/L) to 3.5% at the high dose (10.0 mg Cr(VI)/L). A dose-related increase in plasma chromium began at the 5 mg Cr(VI)/day dose, with 2 subjects not clearly increasing above baseline at either 5 or 10 mg Cr(VI)/day. The RBC chromium

profiles generally mirrored those in plasma. The RBC chromium levels began to decrease within days following cessation of the 10 mg Cr(VI)/L dose and exhibited a rapid and then slow decline which were also similar to that for plasma, with an approximate 50% decrease within 7 days post exposure.

3.3. BIOCHEMISTRY OF INTRACELLULAR HEXAVALENT CHROMIUM

At physiological pH, hexavalent chromium exists in the form of an oxyanion with an overall -2 charge that resembles sulfate and phosphate, and as such, this anion is taken up by cells. This transport system, along with intracellular reduction reactions, allows the anion to accumulate in cells at much higher concentrations than extracellularly. Once hexavalent chromium enters the cell, it is reduced by various intracellular reductants, including ascorbate, glutathione, and cysteine. Low molecular weight thiols (glutathione and cysteine) and ascorbate are believed to be primarily responsible for the intracellular reduction (Suzuki and Fukuda, 1990; Standeven et al., 1991, 1992; Quivryn et al., 2001). Studies on the reduction of Cr(VI) by extracts of rat liver, lung, or kidney have found that ascorbate accounted for at least 80% of Cr(VI) metabolism in these tissues (Standeven et al., 1991,1992). Ascorbate is also the fastest reducer in the in vitro reactions, and its rate of reduction at 1 mM exceeds that of cysteine and glutathione by approximately 13 and 61 times, respectively (Zhitkovich, 2005; Quivryn et al., 2001). This intracellular reduction yields reactive intermediates, chromium(V) and chromium(IV). These reactive intermediates are formed along with oxygen radicals generated via Fenton-like and other possible reactions that occur during intracellular reduction. Depending on the nature of the reducing agent and its concentration, this process can generate various amounts of unstable Cr(V) and Cr(IV) intermediates (Stearns et al., 1994).

Hexavalent chromium taken up by RBCs undergoes reduction to the trivalent form and complexes with Hgb and other intracellular proteins that are sufficiently stable to retain chromium for a substantial fraction of the RBC lifetime. GSH appears to dominate the reduction of hexavalent chromium within RBCs (Wiegand et al., 1984). In RBC suspensions, the addition of GSH results in intracellular reduction of hexavalent chromium to trivalent chromium. The role of GSH was confirmed by decreased chromium binding (from 100 to 40%) in the RBCs following pretreatment with diethylmaleate, a GSH depletion agent (Aaseth et al., 1982). Incubation of human RBCs with $K_2{}^{51}Cr_2O_7$ resulted in depletion of the RBC GSH content to about 10% of normal. Subsequent analysis of RBC lysates suggested that chromium-GSH complexes are formed and that approximately 97% of [^{51}Cr] is bound to Hgb (Wiegand et al., 1984). Excess trivalent chromium in the RBC is sequestered until cell death (Kerger et al., 1997; Aaseth et al., 1982). Over time, the RBC-associated chromium appears to be transferred to the spleen as a result of scavenging of aging RBCs from the blood. Trivalent chromium in plasma does not readily diffuse into RBCs. This explains the observation of lower chromium plasma to RBC ratios following exposure to hexavalent chromium.

Within parenchymal and phagocytic cells, hexavalent chromium may be reduced in the cytosolic and microsomal compartments (De Flora and Wetterhahn, 1989). Isolated liver perfusion in rats suggests that the majority of hexavalent chromium reduction is cytosolic, as 60, 14, 9, and 2% of [^{51}Cr] activity was found in the cytosolic, mitochondrial, microsomal, and nuclear fraction, respectively (Wiegand et al., 1987). Caution should be used in interpreting cell culture data, as the cell culture medium could play a role in hexavalent chromium reduction, confounding the extent of intracellular hexavalent chromium reduction. For example, Dulbeco's Modified Eagle's Medium reduces hexavalent chromium to chromium(V) in the absence of cells (Borthiry et al., 2008). In human bronchial epithelial cells (BEAS-2B), Na_2CrO_4, and to a lesser extent, insoluble Zn_2CrO_4, were reduced to two reactive chromium(V) species; one appeared to be mediated by a thiol-independent NADP(H) reductase, and the other possibly via a hexavalent chromium-GSH intermediate (Borthiry et al., 2008). Electron paramagnetic resonance (EPR) studies of hexavalent chromium reacting with GSH revealed the generation of two reactive chromium(V) intermediates and a GSH thiyl radical (Aiyar et al., 1991). Pulmonary alveolar macrophages (PAMs) also reduce hexavalent chromium via an NADP(H)-dependent reductase and GSH (Petrilli et al., 1986). PAMs in smokers had approximately twice the reductive ability than cells from nonsmokers, ostensibly due to reductase induction by cigarette smoke (Petrilli et al., 1986).

The predominant mechanism for intracellular hexavalent chromium reduction via microsomal enzymes has been extensively described. Incubation of $K_2Cr_2O_7$ with rat liver microsomes or NADP(H) alone resulted in very little hexavalent chromium reduction (Jennette, 1982; Gruber and Jennette, 1978). However, incubation with microsomes and NADP(H) resulted in essentially complete disappearance of hexavalent chromium. Within seconds, hexavalent chromium (as $K_2C_2O_7$) incubated with rat liver microsomes and NADP(H) was reduced to chromium(V), presumably via one-electron transfer from cytochrome P450 (Jennette, 1982).

In contrast to rat liver microsomes, human lung and liver microsomes do not reduce hexavalent chromium via cytochrome P450. Pratt and Myers (1993) showed that human liver and lung microsomes reduced hexavalent chromium via an NADP(H) reductase-dependent system that was not perturbed by the addition of five different P450 inhibitors. The system was, however, inhibited by the addition of $TlCl_3$, indicating the involvement of flavoproteins, specifically cytochrome c reductase. The V_{max} and Michaelis-Menten constant (K_m) values for liver microsomal reduction of hexavalent chromium were 5.03 nmol/minute/mg protein and 1.04 mM, respectively. The human microsomal K_m was 1–3 orders of magnitude lower than those measured in rat liver microsomes (16–34 μM [Mikalsen et al., 1989] to 1.6 mM [Garcia and Jenette, 1981]). Another striking difference between rat and human hexavalent chromium microsomal reduction is the relative insensitivity to O_2 in human microsomes (Pratt and Myers, 1993). While rat microsomal hexavalent chromium reduction was markedly inhibited in the

presence of 0.1% O_2, human microsomal reduction was diminished by only 34–56% in the presence of ambient (21%) O_2. These results suggest two things about the spatial distribution of microsomal hexavalent chromium reduction in rats and humans. First, P450-dependent hexavalent chromium reduction is likely to be confined to the centrilobular region of the rat liver, since an O_2 tension of only 1 mm Hg exists there. Secondly, the insensitivity to O_2 of human microsomes makes it possible for enzymatic reduction to occur in highly aerated tissues, such as the lung.

Myers and Myers (1998) verified and extended the description of enzymatic hexavalent chromium reduction in human liver microsomes. Liver microsomes from five individuals were incubated with Na_2CrO_4 to determine reduction kinetics. Using a series of P450 inhibitors and $TlCl_3$, the authors showed that hexavalent chromium reduction was mediated by flavoproteins, NADP(H)-dependent P450 reductase, and cytochrome b_5. Parameters for reduction kinetics in these five individuals are shown in Table 3-11. The range of V_{max} and K_m values was very similar across subjects. Lung microsomes from one individual exhibited V_{max} and K_m values that were 0.66- and 2.8-fold lower than liver microsome values. Finally, the addition of iron to the liver microsomal system revealed that hexavalent chromium reduction could be stimulated by iron levels that were 3- to 26-fold lower than the hexavalent chromium levels, suggesting that the iron may have a catalytic role in the enzymatic reduction of hexavalent chromium.

Table 3-11. Kinetic parameters of hexavalent chromium reduction in human liver microsomes from five individuals

Parameter	Observation
V_{max}	10.4–10.7
K_m	1.04–1.68
Inhibition by O_2	26–37%
Inhibition by $TlCl_3$	96–100%
Inhibition by P450 inhibitors	
Carbon monoxide	None
Piperonyl butoxide	None
Aminopyrine	None

Source: Myers and Myers (1998).

Proteoliposomes composed of recombinant human P450 reductase and cytochrome b_5 were used to verify that electrons from NADP(H) could be transferred to cytochrome b_5 during the reduction of hexavalent chromium (Jannetto et al., 2001). Markedly less hexavalent chromium reduction occurred in proteoliposomes devoid of cytochrome b_5. Further, hexavalent chromium reduction in proteoliposomes was almost identical to human liver microsomes when corrected for the cytochrome b_5 concentration.

The available data in human and animal studies do not suggest a significant gender difference in metabolism of hexavalent chromium. Further, human liver microsome studies did not identify marked variability in enzymatic rates of hexavalent chromium reduction (Myers and Myers, 1998), although samples were examined from a small number of individuals.

3.4. TOXICOKINETIC CONSIDERATIONS FOR THE MODE OF ACTION OF INGESTED HEXAVALENT CHROMIUM

Consideration of the bioaccessibility, bioavailability, and biochemistry of ingested Cr(VI) are critically important as key toxicokinetic determinants of its mode of action (MOA). Figure 3-6 provides a schematic of the salient features of these processes, and the ensuing text discusses their role in arriving at inferences for the MOA.

As discussed in Sections 2 and 3.1, external reduction in the environment, or bioaccessibility, is a critical factor determining the location, amount, and speciation of a given exposure. The type of compound ingested plays one of the most important roles dictating subsequent toxicity, having a dramatic effect on the digestion, solubilization and speciation of chromium compounds; thus it is a major influence on its internal bioaccessibility, biovailability and toxicokinetics in the body.

Internal bioaccessibility involves factors dictating the extracellular reduction in the GI tract lumen. Physiological fluids in the GI lumen such as gastric juice, and constituents of the diet such as beverages like orange juice, both diminish the uptake of Cr(VI) compounds via their capacities to provide reduction of Cr(VI) to Cr(III). Further, the disposition of chromium in either the trivalent or hexavalent form is strongly dependent on the both the chemical characteristics as well as on the solubility of the chromium compound and its method of administration. Intraindividual variability due to differences in this reduction capacity have been noted in human studies (Kerger et al., 1996; Finley et al., 1997; Paustenbach et al., 1996; O'Flaherty et al., 2001).

The accuracy of any dose-response analysis would be improved by greater rigor afforded the characterization of the reduction capacities, and this may be especially important for interspecies extrapolation. This will likely need to involve a more physiologically-based description of GI uptake as reduction is a function of physiological factors affecting lag time (peristalsis) and spatial distribution in the GI tract. Environmental and internal bioaccessibility processes are both extracellular, in which reduction of Cr(VI) to Cr(III) can occur with the resulting Cr(III) not being able to be actively transported into cells (Levine et al., 2009). Any comprehensive risk modeling must take into account the role of these processes associated with extracellular reduction of Cr(VI) (Zhitkovich, 2005).

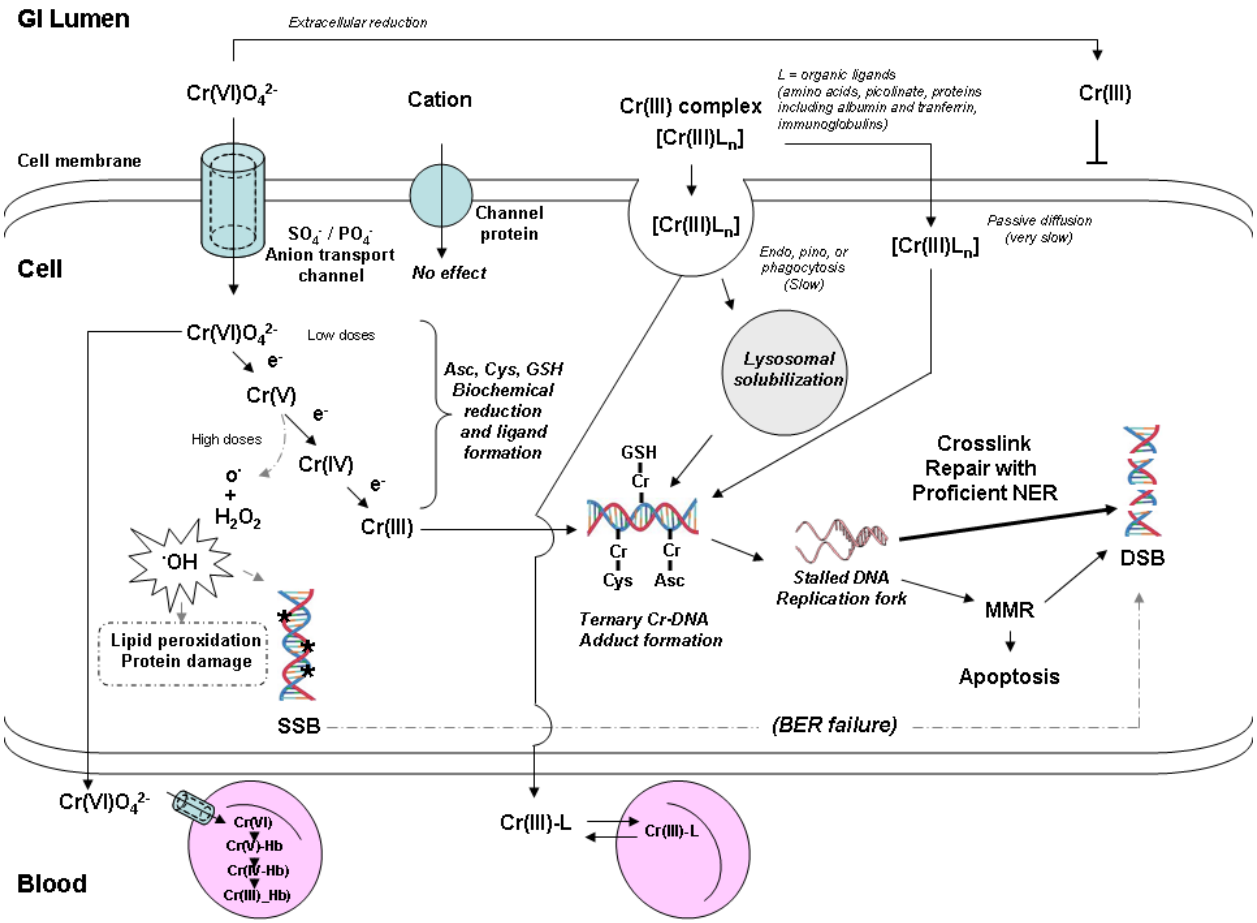
Hexavalent chromium that is not extracellularly reduced is readily transported by sulfate / phosphate anion channels into the GI lumen and due to its reactivity, undergoes biotransformation. As discussed in Section 3.2, Cr(VI) distributes to other tissues, notably the

blood, kidney and liver. The uptake and intracellular reactions depicted and described here for the GI tissue are also applicable to those cells as well.

As described in Section 3.3, Cr(VI) once in the cell, is reduced by various intracellular reductants, including ascorbate, glutathione, and cysteine, lipoic acid, hydrogen peroxide, NAD(P)H-dependent enzymes, fructose and ribose (LeVina et al., 2003, 2007; Nickens et al., 2010). The end product of Cr(VI) intracellular reduction in all biological systems is always Cr(III), but the reduction process can also generate variable amounts of Cr(V), Cr(IV), and organic radicals depending on the nature of the reducing agent, its concentration, and the ratio of reactants (Stearns et al., 1994; Salnikov and Zhitkovich, 2009). The resultant Cr(III) from the intracellular reduction of Cr(VI) forms stable adducts with macromolecules and other cellular constituents.

While the presence of small amounts of short-lived Cr(V) at higher ratios of ascorbate to Cr(VI) can not be unequivocally ruled out due to the technical limitations of the analytical methods employed (electron spin resonance spectroscopy), it is doubtful that environmental levels of Cr(VI) will be sufficient to produce significant quantities of Cr(V) in cells with millimolar ascorbate concentrations (Salnikov and Zhitkovich, 2009). Thus, the reduction reactions to Cr(III)-ligands are depicted as the dominant (solid line) in Figure 3-6, as is the subsequent formation of Cr(III)-DNA adducts, as they have been determined to be mutagenic by the various pathways depicted (as discussed more fully in Section 4). The contribution of oxidative stress and single-strand DNA breaks are depicted to occur at high doses, but the quantitative magnitude for designating “low” versus “high” cannot be established based on the available laboratory animal data.

An additional important note on these biotransformations regards the interpretation and reliability of data from *in vitro* assays. *In vivo,* the intracellular levels of ascorbate are quite high (about 1 mM). In contrast, the levels of ascorbate in tissue culture media are quite low since generally it is not added to the media so that the only source is supplemented fetal bovine serum (FBS). With 10% FBS, the level of ascorbate in tissue cultured cells is only about 50 µM which is 20 times lower than that which is found in vivo (Zhitkovich, 2005). Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006). Attempts to address this concern by delivering ascorbic acid into cultured cells introduce other difficulties because the oxidized dihydro form must be added in order for it to enter the cell (Zhitkovich, 2005).



Source: Adapted from Beyersmann and Hartwig (2008), Salnikow and Zhitkovich (2008), and Holmes (2008).

Figure 3-6. Schematic of ingested Cr(VI) to internal dose in GI tissue and blood. Intracellular molecular mechanisms of biological disposition in GI tissue are expected in other target tissues such as respiratory tract, liver, and kidney. See text for discussion.

3.5. BIOLOGICALLY-BASED MODEL OF INGESTED CHROMIUM COMPOUNDS IN RATS AND HUMANS

Physiologically based pharmacokinetic (PBPK) models are mathematical representations of biological systems in animals and humans that are relevant to the quantitative determination of internal doses of toxic moieties of xenobiotics resulting from external doses or exposures and thereby facilitate interspecies extrapolation (Krishnan et al., 1994). By employing chemical- and species-specific parameter values for tissue volumes, process rates, and reaction kinetics, PBPK models are used to extrapolate internal dosimetry of chemicals across routes of exposure, dose ranges, and species. In risk assessment, the use of PBPK models quantitatively reduces uncertainties in these extrapolations, thus partially or completely obviating the need to apply uncertainty factors (UFs) in the derivation of exposure limits protective of cancer and noncancer effects (Clewell and Andersen, 1985).

The development of PBPK models occurs in four sequential steps: (1) conceptual representation of the body into discrete compartments, (2) parameterization of the model, (3) exercise of the model by simulating one or more exposures and comparing model predictions against empirical observations, and (4) verification of the ability of the model to adequately predict empirical data not used for model exercising (Krishnan and Andersen, 1994). Recent regulatory applications have extended these concepts to use a family approach to evaluate hazard and arrive at risk estimates using a four-step framework for organizing and evaluating toxicity data: 1) exposure, 2) tissue dosimetry, 3) mode of action, and 4) response (Barton et al., 2000). This expansion of the traditional exposure-response analysis has been increasingly utilized in risk assessment and represents advancement for maximal use of designing experiments and maximal end use of toxicity data. The kinetics of a group of metabolically related compounds, i.e., a family, can often be described by development of a template model structure that may only need some refinement in specific parameters to be able to address specific members of that family. The development of the chromium model actually represents such a process and illustrates a distinct advantage of PBPK models, i.e., they can be adaptable to different conditions (e.g., across routes, species, and for both chromium compounds) and provide greater confidence that the physiological basis of the processes are adequately understood and accurately described. This section will briefly describe the development and features of the model described in greater detail elsewhere (O'Flaherty and Radike, 1991; O'Flaherty 1991a,b; O'Flaherty, 1993, 1995, 1996; O'Flaherty et al., 2001), and discuss its potential utility for internal dose descriptions for the assessment of ingested hexavalent chromium.

O'Flaherty published a model for chromium kinetics (both Cr(III) and Cr(VI)) in rats (O'Flaherty, 1996) and humans (O'Flaherty et al., 2001) that was based on a general structure that had been developed for lead kinetics in rats (O'Flaherty, 1991a,b) and humans (O'Flaherty 1993, 1995). Once developed to describe the kinetics of chromium in rats, the model structure was then extended to describe the kinetics of humans using the rat chromium model and the

human lead model. Like lead, chromium exchanges between plasma and the bone surfaces in contact with plasma, and also like lead, although with much lower efficiency, chromium can become incorporated into actively mineralizing bone. Both processes are included in the model. Parallel absorption and disposition schemes for Cr(III) and Cr(VI) are linked in the model by reduction processes occurring throughout the body as well as in the GI tract and in the lungs as points of entry.

Development of the rat model for chromium began with general physiological parameters, as well as those parameters related to body growth and to bone growth and other tissues and organs, as previously defined and assigned in the lead rat model (O'Flaherty, 1991a,b). It was adapted to chromium by first considering the disposition of Cr(III) following an intravenous administration and then introducing other exposure routes in increasing order of kinetic complexity. Incorporation of Cr(VI) kinetics followed a similar strategy. Initial values for each added set of exposure, reduction, or distribution parameters were estimated based on literature data, and model simulations were visually optimized to the appropriate data set at each step in the development process. New model parameters specific to chromium are as follows: rate constant for movement past the GI absorption region, the GI absorption rate constants for both Cr(III) and Cr(VI), clearance constants for passage into and out of tissues including the RBC, the fractional rates of depositon with forming bone, rate constants for the reduction of Cr(VI) to Cr(III) in the GI tract and in tissues, and concentration-dependent urinary clearance consistent with parallel renal processes.

The steps listed below summarize the strategy for development of the rat model and provide an appreciation for the complex considerations of study design and biological processes that were necessary to arrive at the final model structure:

1. General model structure taken from the existing PBPK model for lead (O'Flaherty, 1991a,b) with exclusion of any slow exchange in bone compartment. Relative magnitudes of rapid surface exchange at bone surfaces and formation/resorption of bone in juvenile and mature rats based on visual fit of model to data of Hopkins (1965), a study in which $Cr^{51}Cl_3$ was administered by i.v. and radiolabel monitored for 72 days following injection. The declining body burden data were fit with a three-term sum of exponentials; the third term was presumed to be most closely related to loss of chromium from bone, with bone data reported at 0.25, 4.0 and 24 hours.

2. Extension of time frame of model predictions. Starting value for plasma Cr(III) clearance estimated from chromium body burden data of Hopkins (1965), the second term in that data (described in Step 1) corresponding to a half-life of 5.9 days, results in a clearance rate of 0.025 L Cr/day. Scaling by body weight$^{0.75}$ resulted in whole-body clearance of Cr(III) of 0.055 L Cr/day/kg. This value was later calibrated to be 0.065 to be more consistent with other in vivo data sets after drinking water exposure (e.g., MacKenzie et al., 1959).

3. Refinement of Cr(III) distribution parameters by comparison with data of Visek et al. (1953) on Cr(III) i.v. administration reported at 4 and 42 days.

4. Total clearance of Cr(III) was fractionated into clearances into bile, urine, and GI tract as 1%, 90% of the remainder, and 10% of the remainder, respectively; primarily based on the data of Cikrt and Bencko (1979) who administered Cr(III) salt by i.v. Further work on the model showed that the data of Bragt and Van Dura (1983) and Edel and Sabbioni (1985) were not compatible with significant transintestinal and biliary excretion, so these two fractions were subsequently set at 0 in final form.

5. Addition of RBC compartment in communication with arterial blood. The first-order rate constants for loss of Cr(III) from RBC to plasma and transfer of Cr(III) from plasma to the RBC were fixed on the basis of measured half-life of chromium association with RBC (Bishop and Surgenor, 1964). These were not changed in further model development. Estimates of the rate constant for transfer of Cr(VI) from plasma to RBC based on in vitro data on human RBC suspended in either saline (Gray and Sterling, 1950) or blood plasma (Weigand et al., 1985). The same half-life was initially used for transfer of Cr(VI) between plasma and peripheral tissues, but these were changed for poorly perfused tissues in order to fit the data of Weber (1983).

6. Total excretion clearance for Cr(VI) set and changed as above in Step 4 for Cr(III).

7. Percent of chromium dose excreted in the urine in 24 hour following i.v. injection of a soluble salt of either Cr(III) or Cr(VI) in rats was reported by Cikrt and Bencko (1979) to be independent of oxidation state of administered compound. Initial estimate of excretion clearance of Cr(VI) was set equal to Cr(III) and the two values remained equal in the course of model development.

8. Link of Cr(VI) model to Cr(III) model by reduction processes in all tissues. A single value of the first-order reduction rate constant was provisionally assigned to the Cr(VI) pools in the RBCs, peripheral tissues, and lung. This simplification proved satisfactory and was retained in the final model. The value of the reduction rate constant was determined by fitting the tissue concentration data of Weber (1983) in accordance with the results of studies in which little or no reduction was observed in human plasma in vitro (Gray and Sterling, 1950; Korallus et al., 1984).

9 Expansion of i.v. model to include GI uptake. First-order rate constant set at 0.01 per day for GI absorption of Cr(III) estimated from the single bolus dose data of MacKenzie et al. (1959) at 1.8%, shown to be in agreement with the estimate of Mertz et al. (1965) at 2–3% for oral administration. The same study found 85% of an orally administered soluble Cr(VI) salt had been reduced before it could reach the intestinal absorption site. Model was run with inclusion of reduction pathway and the first-order reaction rate constant set to give 85% reduction and 10% absorption.

10. Final structural development step was to expand the composite model to allow absorption and elimination of chromium from the lung. Pulmonary clearance of Cr(VI) salts is not dose-dependent within a reasonable dose range (Weber, 1983; Bragt and van Dura, 1983). Mucociliary transfer to the GI tract identified as second route of chromium clearance. Both Cr(III) and Cr(VI) assigned to two lung pools in the model. Chromium

from either intratracheal (i.t.) or inhalation exposures enters the first pool, from which it can be systemically absorbed, transferred to the second pool, or cleared by mucociliary action. Chromium in the second pool can be only cleared by mucociliary transport.

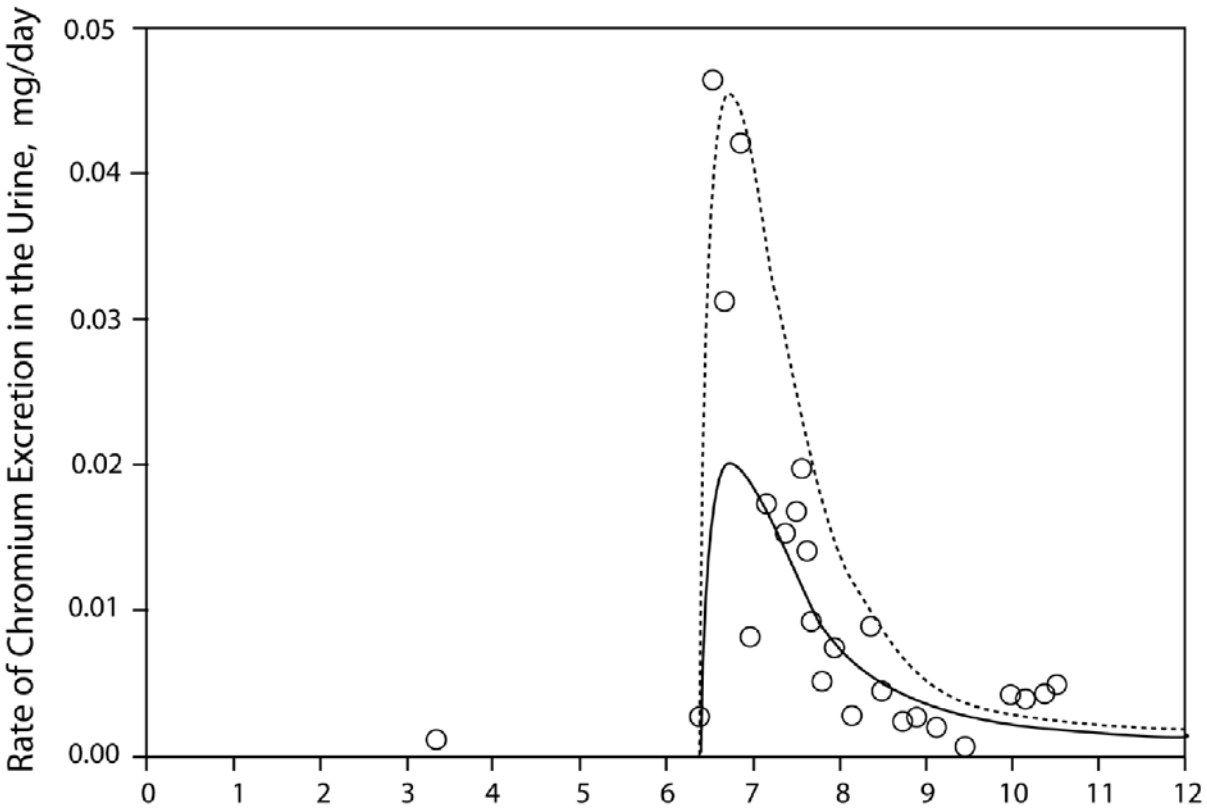
11. Adjustment of initial model parameter values by visual optimization to fit data from O'Flaherty and Radike (1991). Published parameter estimates, especially for tissue uptake and loss were refined by optimization of model predictions to the data set of Weber (1983) in conjunction with the data sets of Bragt and Van Dura (1983) and Edel and Sabbioni (1985). All were studies of radiolabeled chromium following i.t. administration of soluble ^{51}Cr(VI), or in one instance ^{51}Cr(III), salts. The Weber (1983) data set consisted of time course data in several tissues, whole blood, plasma, GI tract, and carcass for 42 days after a single i.t. dose. The Bragt and Van Dura and Edel and Sabbioni data sets had only limited tissue concentration measurements, but extensive measurements of cumulative excretion in urine and feces.

To test the generalizeability of the final form of the model, the inhalation study of Langard et al. (1978) was simulated. The study of Langard et al. (1978) consisted of a series of inhalation exposures of rats to zinc chromate dust, 6 hour/day for 4 days followed by 4 days during which urinary excretion was monitored and 37 days during which blood chromium levels were monitored. Agreement of the O'Flaherty model against these data was reasonably good despite the route extrapolation required. However, the model only fit the data of the MacKenzie et al. (1958) drinking water study moderately well, as the nonlinear kidney and liver oncentrations were over predicted. Modification of the uptake description to include a Michaelis-Menton term may address this issue (see below).

The human model (O'Flaherty, 2001) was developed based on the rat using appropriate scaling of physiological parameters and by estimating specific chromium-related parameters using several studies in adult human volunteers administered chromium in drinking water (described in Section 3.2 [Finley et al., 1997; Kerger et al., 1996; Paustenbach et al., 1996]). Default values, determined by gender and age, were used for their body weights. The studies of Kerger et al. (1996) and Finley et al. (1997) were used for calibration of the chromium-specific parameters (e.g., clearance constants) in the model structure and the Paustenbach et al. (1996) study was used for verification. In the absence of data on the magnitude of the rate of deposition of Cr(III) or Cr(VI) in human bone, the fractional rates of deposition were assigned the values of 5 for Cr(III) and 15 for Cr(VI), the same values used in the rat model.

The human model generated reasonable time profiles to the data of Paustenbach et al. (1996) despite the variability of the urinary excretion rates. Figure 3-7 illustrates the dependence of the urinary excretion on the form of the chromium administered. As discussed for the data of Kerger et al. (1996), when chromium has been administered as Cr(III) citrate rather than as the inorganic salts of $CrCl_3$ or $K_2Cr_2O_7$, transfer of chromium from the blood to a compartment from which excretion occurs is favored relative to other tissues. As a result, when the $CrCl_3$ clearance curve is applied and the excretion rates were optimized to fit urinary excretion rates, plasma

concentrations in that same subject were greatly over-predicted; if the model had instead been optimized to plasma concentrations, urinary excretion rates would have been underpredicted. O'Flaherty revised the clearance expression to address the administration in orange juice. The alternate expression gives clearances of 2 L/day at chromium concentrations within the background concentration range of 0.05–0.15 µg/L and is represented by the dotted line in Figure 3-7. The fit to the data for the model prediction of the kinetic behavior of chromium in the plasma for the Cr(VI)-OJ salt was greatly improved.



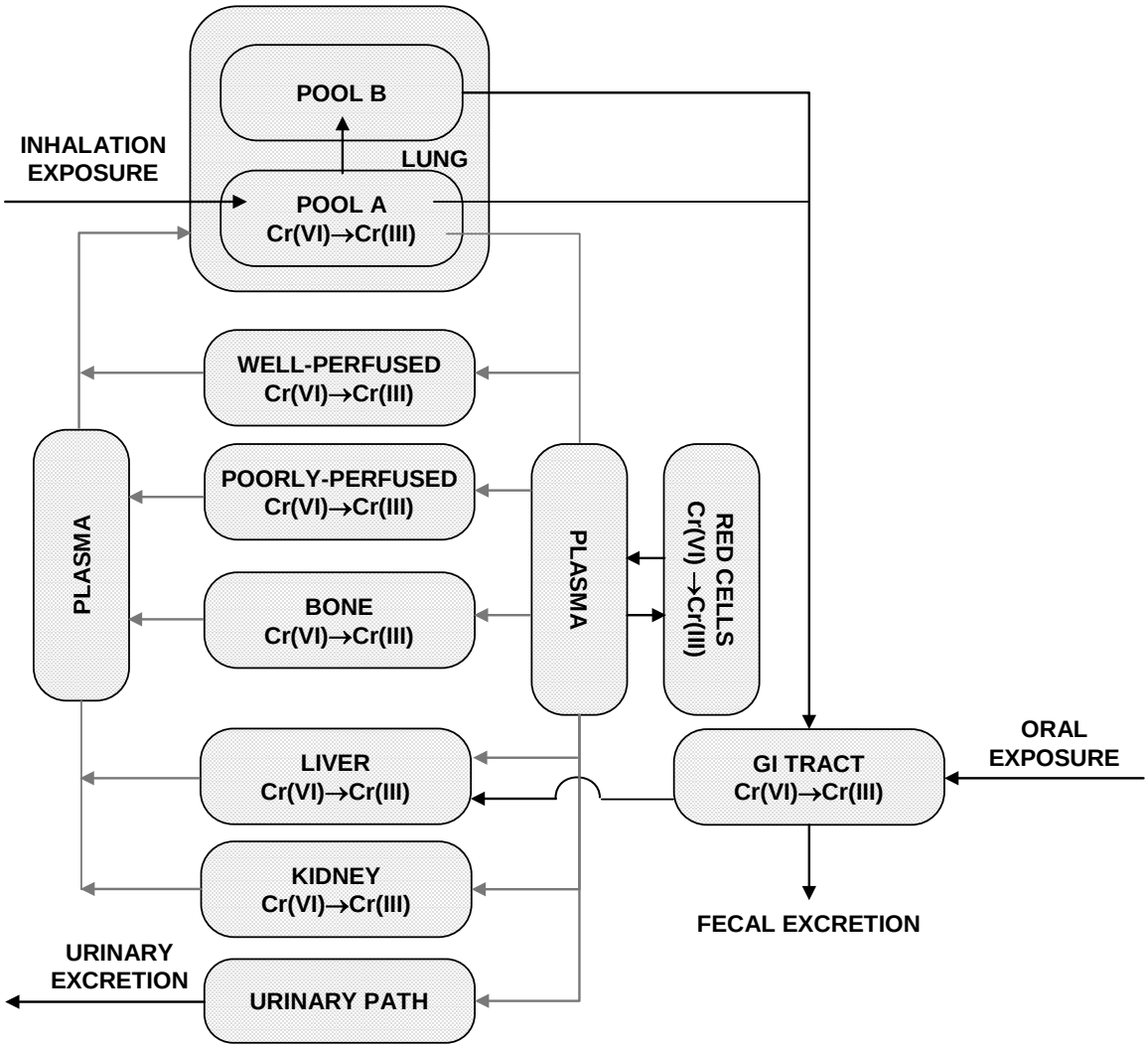
Source: O'Flaherty et al. (2001).

Figure 3-7. Observed and simulated plasma chromium concentrations predicted by the PBPK model for a human subject ingesting Cr(VI) dissolved in orange juice (CrVI-OJ) in the study of Kerger et al. (1996). The solid line is the simulation using the general model expression for urinary clearance. The dotted line represents a simulation using an alternate expression for that clearance (see text).

Another key finding of the model simulations was that based on total urinary excretion, a consistently greater percentage of Cr(VI) than of Cr(III) is absorbed. This implies that some Cr(VI) escaped reduction in the stomach and entered portal venous blood. This greater absorption of Cr(VI) versus Cr(III) does not imply that the reduction capacity of gastric juice was exceeded, but rather that absorption from the GI tract is so rapid that it is able to compete effectively with reduction in the stomach (O'Flaherty, 1996). The rapidity of absorption is further supported by the rate at which chromium appears in the blood.

The model schematic is provided in Figure 3-8 and the key parameters for both the rat and human models are provided in Table 3-12. The model accounts for both oral and inhalation exposures, plasma and RBC distribution, distribution in key target tissues such as liver and kidney, and elimination via urine and feces. Additional target tissues could be defined from the well- and poorly-perfused tissues if required; likewise, a more complex respiratory or GI tract description could be incorporated as needed (see below).

As with all models of this type, certain parameter values are highly correlated. For several constellations of constants that define parallel and competing processes, the relative values of the constants are more important than are their absolute values in order to achieve a good fit to the concentration data. These groups of constants include those determining the simultaneous rapid excretion of Cr(VI) and its uptake into tissue, those determining loss of Cr(III) from tissues, and those determining the relative rates of Cr(VI) reduction in the RBC and release from the RBC. Uptake of Cr(III) into tissues has relatively little impact on the predictions of the model (O'Flaherty, 1996).



Source: O'Flaherty (1996).

Figure 3-8. Schematic of structure for PBPK model of chromium in the rat and human.

Table 3-12. Chemical-specific parameters in the rat and human chromium models

Parameter[a]	Rat		Human		Definition
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	
Absorption					
KGI	0.01	0.04	0.25	2.5	First-order rate constant for absorption from the GI tract (Da^{-1})
KLU	0.2	2.0	NA	NA	First-order rate constant for absorption from the bioavailable lung pool (pool A) (Da^{-1})
KMUCOA	0.8	0.8	NA	NA	First-order rate constant for mucociliary clearance from pool A to the GI tract (Da^{-1})
KMUCOB	0.025	0.025	NA	NA	First-order rate constant for mucociliary clearance from the nonbioavailable lung pool (pool B) to the GI tract (Da^{-1})
KLUAB	1.2	1.2	NA	NA	First-order rate constant for transfer from pool A to pool B (Da^{-1})
FRLUNG	NA	NA	0.3	0.3	Fraction of inhaled chromium absorbed to blood
FRTRGI	NA	NA	0.7	0.7	Fraction of inhaled chromium transferred to GI tract
Distribution					
CR	5.0	15.0	NA[b]	NA[b]	Relative clearance of chromium into mineralizing bone (L blood plasma cleared/L new bone formed)
KINRBC	0.0003	1.5	12.0	NA	Clearance from plasma to red cell (L/Da)
KDIN	0.007	1.5	3.0	30.0	Clearance from plasma to kidney (L/Da)
LDIN	0.0001	1.5	3.0	30.0	Clearance from plasma to liver (L/Da)
WDIN	0.0001	1.5	3.0	30.0	Clearance from plasma to other well-perfused tissues (L/Da)
PDIN	0.0001	0.01	3.0	30.0	Clearance from plasma to poorly-perfused tissues (L/Da)
BDIN	0.0001	0.01	NA[b]	NA[b]	Clearance from plasma to bone (L/Da)
CR	NA	NA	5.0	15.0	Fraction deposition from blood to forming bone
KOUTRBC	0.0003	10.0	12.0	NA	Clearance from red cell to plasma (L/Da)
KDOUT	0.001	10.0	3.0	30.0	Clearance from kidney to plasma (L/Da)
LDOUT	0.0003	10.0	3.0	30.0	Clearance from liver to plasma (L/Da)
WDOUT	0.001	10.0	3.0	30.0	Clearance from other well-perfused tissues to plasma (L/Da)
PDOUT	0.003	10.0	3.0	30.0	Clearance from poorly perfused tissues to plasma (L/Da)
BDOUT	0.003	10.0	NA[b]	NA[b]	Clearance from bone to plasma (L/Da)
Excretion					
KFX	1.5	1.5	14.0	14.0	First-order rate constant for loss of chromium from intestinal tract contents to the feces (Da^{-1})
QEC	0.065	0.065	NA[c]	NA[c]	Excretion clearance from the plasma (urinary clearance) (L/kg/Da)
CLEAR[c]	NA	NA	12.0	12.0	Parameter in expression for clearance from blood plasma to urine (L/d)
MAX[c]	NA	NA	0.008	0.008	Parameter in expression for clearance from blood plasma to urine (mg/d)
KM[c]	NA	NA	0.0008	0.0008	Parameter in expression for clearance from blood plasma to urine (mg/L)
FB	0.0	0.0	NA	NA	Fraction of body burden secreted in the bile
FI	0.0	0.0	NA	NA	Fraction of body burden excreted via the GI tract

Table 3-12. Chemical-specific parameters in the rat and human chromium models

Parameter[a]	Rat		Human		Definition
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	
Reduction					
KREDRC	NA	0.7	NA	7.0	First-order rate constant for reduction of hexavalent chromium to Cr(III) in the red cell (Da^{-1})
KREDBP	NA	NA	NA	0.2	First-order rate constant for reduction of hexavalent chromium to Cr(III) in blood plasma (Da^{-1})
KREDKL	NA	NA	NA	500.0	First-order rate constant for reduction of hexavalent chromium to Cr(III) in kidney (Da^{-1})
KREDGI	NA	10.0	NA	100.0	First-order rate constant for reduction of hexavalent chromium to Cr(III) in GI tract contents (Da^{-1})
KRED	NA	0.5	NA	5.0	First-order rate constant for reduction of hexavalent chromium to Cr(III) in all other tissues and in lung contents (Da^{-1})
Lag time for excretion of urine					
FRHOLD	0.7	0.7	NA	NA	Fraction of urinary chromium not excreted immediately; that is, temporarily held in pool
KHOLD	0.05	0.05	NA	NA	First-order rate constant for excretion from the retained urine pool (Da^{-1})
FR	0.10	0.10	NA	NA	Fraction of chromium in retained urine that is associated with the kidney

[a]Parameter names are those for human model in cases where the reported rat and human parameter names were not identical.
[b]Exchanges between blood plasma and cortical and trabecular bone are simulated as functions of bone formation and resorption rates.
[c]

$QE = CLEAR - \frac{MAX}{KM + CBP}$, where QE is clearance from blood plasma to urine (L/d) and CBP is plasma concentration of chromium (mg/L).

NA = not applicable

Sources: O'Flaherty (1996) (rat parameters); O'Flaherty et al. (2001) (human parameters).

The model behavior in several respects suggests that it reflects critical physiological behavior with reasonable accuracy. For example, although uptake of Cr(VI) into tissues is clearly more rapid than that of Cr(III), it is not possible to fit Cr(VI) clearance disposition data satisfactorily using an excretion clearance greater than that assigned to Cr(III). That is, the mechanism of tissue uptake is fundamentally different than that for excretion, which would be expected if one was carrier-mediated and the other not. Additionally, the magnitudes of the observed tissue chromium concentrations require that a significant portion of tissue uptake be chromium as Cr(VI), which is reduced and trapped as Cr(III), especially in the RBC. Cr(III) would not have entered the RBC sufficiently rapidly to achieve the observed concentraions. Further, the transient early chromium concentration peaks seen in certain tissues (e.g., liver) would not be adequately described by assuming only that some Cr(VI) taken up by the tissue is subsequently lost as Cr(VI).

As illustrated above, O'Flaherty (1996, 2001) noted for both models that a key uncertainty in the models is that there is limited information regarding the factional absorption from environmental sources. Since even the soluble salts of Cr(III) and Cr(VI) are not particularly well absorbed from either the GI tract or respiratory tract, bioaccessibility of chromium to absorption processes will prove to be the most important single characteristic determining its potential absorption and toxicity. The urinary clearance observed in studies in which inorganic Cr(VI) or Cr(III) were administered differ from the urinary clearance of Cr(III) administered as a complex with citric acid anions. The latter are consistent with chromium clearances reported in the general population. The disposition of chromium in either the trivalent or hexavalent form was strongly dependent on both the chemical characteristics as well as on the solubility of the chromium compound and its method of administration. Studies in which the chromium compound was given by i.v., intramuscular (i.m.), or i.p. injection do not generate data on which an understanding of the kinetics of chromium can be based and thus are not suitable for setting chromium-exposure standards.

A second uncertainty noted by O'Flaherty (1996) was that little is known about the importance of bone as a reservoir and continuing source of internal exposure. Dependence of bone chromium uptake on age and physiologic status may be important features to complete any comprehensive model application to chromium kinetics in populations relevant to risk assessments.

Despite these limitations, the human model in its present form could be useable for the generation of rough estimates with a urinary clearance set to a constant value of 1–2 L/day and the rate constants of GI absorption set at 0.25/day for Cr(III) and 2.5/day for Cr(VI), i.e., 10 times the value of Cr(III). However, neither the rat nor human version of the model in its present form has been subjected to formal computerized optimization of parameter values. As suggested by O'Flaherty and Radike (1991), its use for predictive applications in risk assessment would be greatly enhanced by both a sensitivity analysis and optimization of key parameters, with

attention to the variety, reliability, and utility of the kinetic data available. Newer data sets published since the model was published may be particularly informative to this exercise, including those of Kargacin et al. (1993) in mice and rats, Sutherland et al. (2000) in rats, and the NTP (2008) bioassay in rats and mice.

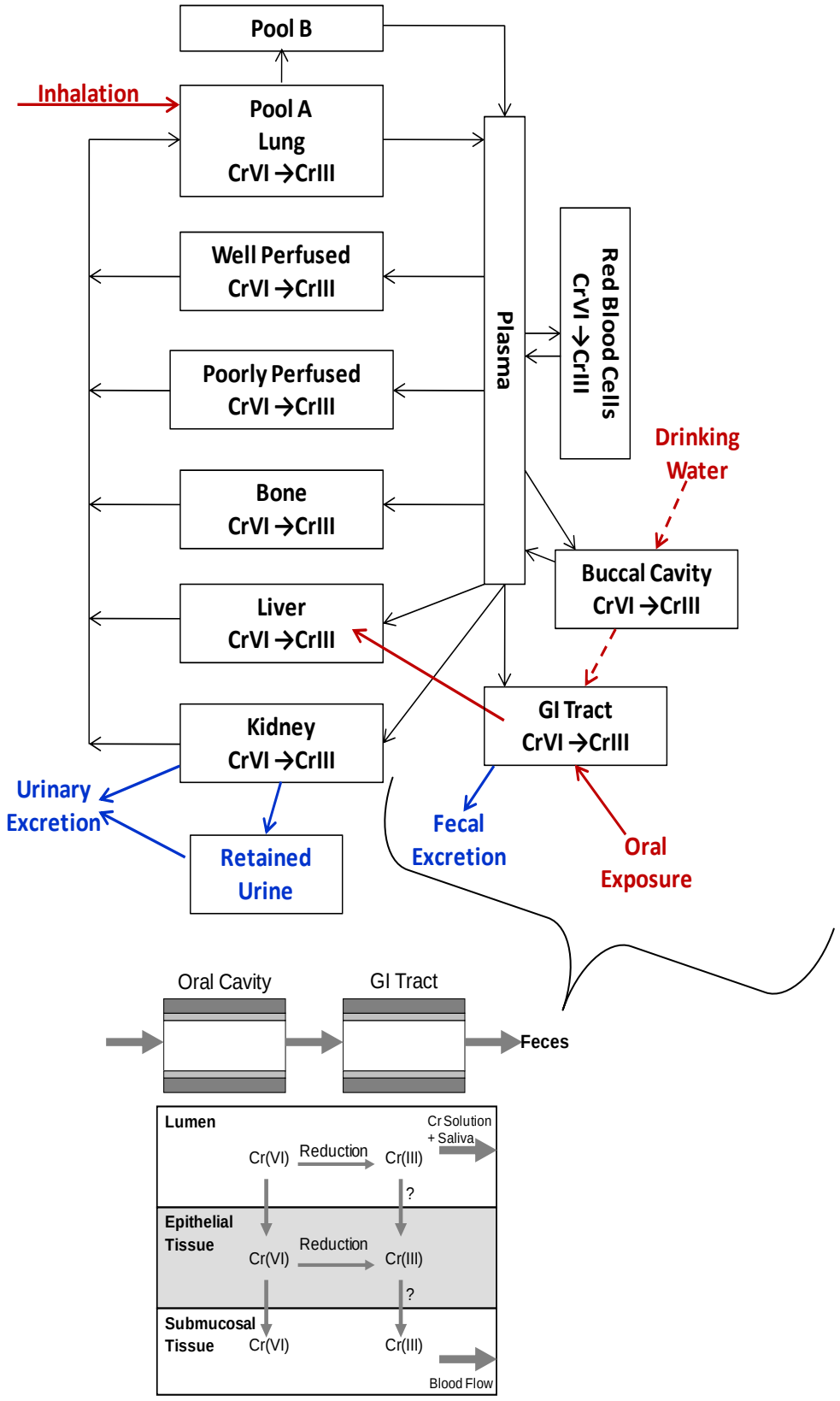
Because of the toxicity and tumors observed in the buccal cavity of rats and the duodenum in mice of the recent NTP 2-year oral bioassay with sodium dichromate dihydrate in drinking water (NTP, 2008; Stout et al., 2009), new effort has been devoted to extending the O'Flaherty model to predict kinetics in those tissues for those species (Campbell et al., 2009). The O'Flaherty rat model was extrapolated to the mouse using known physiological parameter values and allometric scaling of chromium kinetic parameters (Table 3-13).

Table 3-13. Parameters for mouse developed by Campbell et al. (2009)

Parameter	Mouse	Rat	
QCC	16.5	15.0	Cardiac output (L/h/kg)
Fraction of cardiac output			
QLC	0.02	0.25	Liver
QKC	0.17	0.17	Kidney
QIC	0.141	0.17	GI tract
QBC	0.03	0.03	Bone
QOCC	0.01	0.10	Oral cavity
Oral cavity			
SAOC	2.5	5	Surface areas (cm^2)
VLOC	1.2	2.4	Volumes (mL)
WLO	0.006	0.006	Lumen thickness (cm)
WTO	0.01	0.01	Tissue thickness (cm)
WXO	0.02	0.02	Submucosal thickness (cm)
FRACS	0.43	0.43	Saliva flow (fraction of ingestion rate)
Tissue volume (fraction of body weight)			
VLC	0.055	0.04	Liver
VKC	0.0167	0.01	Kidney
VIC	0.0422	0.03	GI tract
VRBCC	0.03	0.03	RBC
VWC	0.2	0.2	Well perfused (less liver, kidney, and GI tract)
VPC	0.7	0.7	Slowly perfused (less bone)

Source: Campbell et al. (2009).

An oral mucosa compartment was added to the rat model and compartments for the stomach, small intestine, and large intestine were added to the model description as shown in the schematic of the model in Figure 3-9. This structure is likely superior to the simplified first-order process used by O'Flaherty in that it addresses proximal to distal movement of chromium through the GI tract. Using the NTP (2007) subchronic data, uptake in the model was fit to the kidney data and then used to predict the blood uptake and GI tissue distribution of total chromium in the NTP subchronic kinetic studies for the rats and mice. The apparent nonlinear, dose-dependent uptake of chromium from drinking water in these studies could be adequately described using Michaelis-Menten kinetics (Campbell et al., 2009). The model predictions were shown to adequately fit the kidney and blood data. Further development, optimization, and publication of the model against additional data sets with richer tissue information such as Kargacin et al. (1993), Sutherland et al. (2000), and the NTP (2008) is warranted. Advantages of this type of PBPK modeling is that the nonlinearities and spatial properties in uptake process can be described more physiologically, quantitative extrapolations can be performed across species, and predictions of target tissue dose can provide a more accurate description of dose-response.



Source: Campbell et al. (2009).

Figure 3-9. Extended PBPK model structure to predict chromium distribution in the oral cavity and GI tract tissue for rats and mice.

3.6. CONSIDERATION OF CHROMIUM ESSENTIALITY VERSUS TOXICITY

Further research is needed on this important area for characterizing the beneficial versus toxic effects of chromium (Vincent, 2004; Costa and Klein, 2006). Chromium trivalent supplements are among the most highly absorbed forms of Cr(III). Chromium (III) has been purported to have beneficial effects on insulin, but that role is currently debated (Costa and Klein, 2006; Stearns, 2000). It is now believed that "nutritional supplement" may be a better term for describing the biological role for Cr(III) since the generation of chromium deficiency in humans is difficult to define (Mertz, 1998; Stearns, 2000; Costa and Klein, 2004). As evidenced in the preceding sections, any discussion of the potential toxicity of Cr(III) should also include an evaluation of Cr(VI) as the biological disposition of the two are related (Stearns, 2000). The Reference Daily Intake (RDI) for chromium of 50–200 µg was revised to a Dietary Reference Intake (DRI) of 35 µg. DRI values are the most recent set of dietary recommendations established by the Food and Nutrition Board of the Institute of Medicine, 1997-2001. They replace previous RDAs, and may be the basis for eventually updating the RDIs.

4. HAZARD IDENTIFICATION

4.1. ORAL STUDIES IN HUMANS

The human health effects observed following oral ingestion of hexavalent chromium usually come from individuals accidentally or intentionally ingesting hexavalent chromium compounds or from human populations unknowingly consuming food or drinking water contaminated with hexavalent chromium.

4.1.1. Acute Exposure

Several case reports have been published on clinical signs and symptoms in individuals following acute accidental or intentional ingestion of high doses (fatal or near fatal) of hexavalent chromium compounds, including chromic acid (Loubieres et al., 1999; Saryan and Reedy, 1988; Fristedt et al., 1965), potassium dichromate (Hantson et al., 2005; Clochesy, 1984; Iserson et al., 1983; Sharma et al., 1978; Kaufman et al., 1970; Partington, 1950; Goldman and Karotkin, 1935), and ammonium dichromate (Hasan, 2007; Reichelderfer, 1968). Clinical presentation of patients following acute, high-dose exposure was similar, regardless of the specific hexavalent chromium compound ingested, and included the following: abdominal pain, nausea, and vomiting; hematemesis and bloody diarrhea; caustic burns of mouth, pharynx, esophagus, stomach, and duodenum and GI hemorrhage; anemia, decreased blood Hgb, abnormal erythrocytes, and intravascular hemolysis; hepatotoxicity (hepatomegaly, jaundice, elevated blood bilirubin, and liver enzymes activities); renal failure (oliguria and anuria); cyanosis; and metabolic acidosis, hypotension, and shock. Findings on tissue biopsies included hepatic fatty degeneration and necrosis and renal tubular degeneration and necrosis (Loubieres et al., 1999; Sharma et al., 1978; Kaufman et al., 1970; Reichelderfer, 1968). Based on estimated amounts of hexavalent chromium ingested, the range of lethal doses for hexavalent chromium in humans is from approximately 4.1 to 357 mg hexavalent chromium/kg body weight (Loubieres et al., 1999; Saryan and Reedy, 1988; Clochesy, 1984; Iserson et al., 1983; Kaufman et al., 1970).

A series of acute and short-term repeated (17-day) ingestion studies were conducted on volunteers to evaluate hexavalent chromium pharmacokinetics (Corbett et al., 1997; Finley et al., 1997; Kerger et al., 1997, 1996b; Kuykendall et al., 1996; Paustenbach et al., 1996). With the exception of Paustenbach et al. (1996), these studies reported that study protocols were reviewed and approved by a human use committee comprised of three board-certified occupational physicians and one board-certified toxicologist. In each case, the committee determined that participants were properly informed of the reported adverse health effects associated with hexavalent chromium exposure. The study by Paustenbach et al. (1996) involved a single male volunteer. The methods section of this study noted that “The volunteer had a PhD in toxicology,

and the test protocol was approved by a human use committee." As part of these studies, standard clinical evaluations were performed that included blood cell counts, blood clinical chemistry (SMA-20), and urinalysis (volume, specific gravity, creatinine). In the longest duration exposure, a single subject ingested 2 L/day of a solution of containing 2 mg hexavalent chromium/L (as potassium dichromate in water) for 17 consecutive days (approximately 0.06 mg hexavalent chromium/kg-day, assuming a 70-kg body weight) (Paustenbach et al., 1996). In shorter duration studies, 3–5 subjects ingested 1 L/day of solutions containing 0.1–10 mg hexavalent chromium/L in water (approximately 0.001–0.14 mg hexavalent chromium/kg-day, assuming a 70-kg body weight) for 1–3 days (Finley et al., 1997; Kerger et al., 1997, 1996b; Kuykendall et al., 1996). Data from the clinical evaluations were not reported; however, results were described in general terms that suggested that values for clinical chemistry endpoints were "similar" when measured prior to, during, or following dosing (Paustenbach et al., 2003, 1996).

4.1.2. Environmental Exposure

Human studies of possible associations between oral exposures to environmental hexavalent chromium and health outcomes include several epidemiology studies in which health outcomes (primarily cancer) were evaluated among populations that resided near sources of industrial waste containing hexavalent chromium compounds in Liaoning Province, China (Kerger et al., 2009; Beaumont et al., 2008; Zhang and Li, 1997, 1987, 1980), Kings County/San Bernardino County, California (Fryzek et al., 2001), Nebraska (Bednar and Kies, 1991), and Glasgow, United Kingdom (Eizaguirre-Garcia et al., 2000, 1999). In addition to these studies, two cases of Hodgkin's disease in residents of Hinkley, California, where hexavalent chromium was used as a cooling additive at a local gas plant, were described in a case report by Bick et al. (1996).

Liaoning Province, China (Kerger et al., 2009; Beaumont et al., 2008; Zhang and Li, 1997, 1987)

In 1987, Zhang and Li published a paper describing the soil and water contamination by chromium in the vicinity of an alloy plant where chromium was smelted in the JinZhou area of Liaoning Province, China (Zhang and Li, 1987). This paper was based on an earlier unpublished report (Zhang and Li, 1980). A more detailed mortality analysis, which included variation in cancer mortality rates among the five villages along the Nuer River, was published in 1997 (Zhang and Li, 1997) in the *Journal of Occupational and Environmental Medicine.* This study has had a controversial history that culminated in the retraction, in 2006, of the latest report (Zhang and Li, 1997) by the editors of the *Journal of Occupational and Environmental Medicine* because "financial and intellectual input to the paper by outside parties was not disclosed" (Brandt-Rauf, 2006). The financial and intellectual input in question were those from a consulting firm that had (or may have had) financial ties with industry clients potentially liable

for chromium wastes in the United States (Smith, 2008). Two reanalyses of data compiled by Zhang and Li have also been reported (Kerger et al., 2009; Beaumont et al., 2008). The following presentation of the studies begins with a description of the geographic area, industrial operations, and resulting chromium dispersion in the surrounding communities, with information obtained from the most recent reports (Kerger et al. 2009; Beaumont et al., 2008) and from earlier published and unpublished reports (Zhang and Li, 1986, 1980; JinZhou Antiepidemic Station, 1979). The commonalities and differences in the reanalyses by Kerger et al. (2009) and Beaumont et al. (2008) are then described.

The study area is west of JinZhou, a city in Liaoning Province in northeastern China. This area was described by Zhang and Li (1987) as being primarily agricultural with some pockets of industries. One of the industrial plants is the JinZhou ferrochromium alloy plant, located near the Nuer River. The town of TangHeZi was developed around the plant (Zhang and Li, 1980). A series of five small rural villages (JinChangBao, Nuer River Village, YangXing, ShiLiTai, and WenJiaTun) are located approximately 1–5 km to the east of the plant along the Nuer River. The alloy plant began trial smelting of chromium in 1959, small-scale production in 1961, and mass production in 1965 (Zhang and Li, 1987). Liquid wastes from the production process were released to a dry river bed (the "Old Nuer River") near the plant. The amount of hexavalent chromium in the wastewater was considerable (estimated as 20 mg/L at the end of the discharge pipe) (Zhang and Li, 1986). Solid wastes (>300,000 tons by 1986) were stored in outdoor piles and were subject to leaching to surface water and groundwater. These piles of ore residue were the main long-term source of underground water contamination. Hexavalent chromium was also released into the air through the various production and waste processes, with a northeast prevailing wind pattern. An additional source of chromium exposure was from food grown in areas using contaminated well water for irrigation.

In 1964, residents in the Nuer River Village noticed a yellowing of the color of their drinking water. The local health department (referred to as the "JinZhou Disease Control and Prevention Station", "JinZhou Health and Anti-epidemic Station", or "JinZhou Antiepidemic Station" depending on the translation) initiated testing of well water samples in each of the five villages in 1965. Chromium was found in 75 (28%) of the first set of samples from 266 wells in JinChangBao and Nuer River Village, with levels up to 10 mg/L. By the end of 1965, the zone of underground water contamination had spread, following a path eastward from the plant. In JinChangBao, 41% of the wells contained hexavalent chromium, as did 96% of the wells in Nuer River Village. The highest concentration (5 mg/L) was found in YangXing and Nuer Railway Station, which are east of JinChangBao and Nuer River Village. In 1966, hexavalent chromium was detected in the Nanshan reservoir (supplying drinking water to JinZhou), 9 km from the alloy plant. Monitoring of well water continued, and the expansion of the contamination zone appeared to peak in 1979 (Zhang and Li, 1986). A variety of efforts to reduce the chromium run-off were undertaken in 1965–1967.

Table 4-1 includes a compilation of the available data from the 1965 water sampling studies (based on Table 2 from Beaumont et al., 2008, with the addition of the distance from the plant and average chromium levels in the well water samples from Kerger et al., 2009). The analytical methods used to quantify chromium were not reported, but these values (and all other values for chromium concentrations noted below) were reported as hexavalent chromium; Beaumont et al. (2008) note that other forms would not be expected to be water soluble. Beaumont et al. (2008) and Kerger et al. (2009) are in general agreement regarding their interpretation of the 1965 water testing data. There is disagreement, however, as to what can be established regarding levels in later years (Table 4-1), and the stability of the relative levels among the villages. Beaumont et al. (2008) do not consider the available data to be adequate to classify the individual villages with respect to a relative ranking of exposure, given the lack of information regarding the selection of wells sampled, lack of information regarding use of specific wells by individuals within the villages, paucity of data from later years, and rapid changes in chromium concentrations in various areas due to the groundwater movement as well as the efforts to curtail the chromium contamination. Kerger et al. (2009), however, use the 1965 well water sample data to derive two measures of exposure (average chromium concentration and percent of wells >0.05 mg/L) that they applied to each of the five villages for an exposure-response analysis of cancer risk.

Table 4-1. Data pertaining to hexavalent chromium concentrations in drinking water in five villages along a path of groundwater contamination from an alloy plant in western JinZhou, China from 1965 to 1979

	Village (km from alloy plant)				
Yr	**JinChanBao (1.4)**	**Nuer River Village (1.5)**	**YangXing (3.0)**	**ShiLiTai (3.5)**	**WenJiaTun (5.0)**
Early 1965[a]	Hexavalent chromium detected in 75 (28%) of 265 wells sampled in JinChanBao and Nuer River Village; 73 of the 75 wells were in Nuer River Village; 41 (15%) were >2.0 mg/L (range 0.6–10.0 mg/L).				
Later in 1965[a,b]					
Number of wells sampled[a,b]	123	170	50	21	33
Hexavalent chromium (mg/L)[a]	Number of wells (%)				
<0.001	73 (59)	7 (4)	14 (28)	2 (10)	27 (82)
0.001–<0.05	35 (28)	1 (1)	16 (32)	19 (90)	6 (18)
0.05–<0.1	7 (6)	5 (3)	5 (10)	0 (0)	0 (0)
0.01–<0.5	8 (7)	27 (16)	12 (24)	0 (0)	0 (0)
0.5–<1.0	0 (0)	17 (10)	2 (4)	0 (0)	0 (0)
1.0–<5.0	0 (0)	76 (45)	1 (2)	0 (0)	0 (0)
≥5.0	0 (0)	37 (22)	0 (0)	0 (0)	0 (0)
Maximum (mg/L)[a,b]	0.4	20.0	<5	<0.05	<0.05
Average (mg/L)[b]	0.031	2.6	0.18	0.02	0.004
1966[c]			0.002–20.0		
1967[b]			<0.05	<0.05	<0.05
1972[b]				<0.05	
1974	10.5[d]		0.01–0.05[c]		
1979[c]	0.06–4.33		0.001–0.03		0.003–0.004

[a]As reported by Beaumont et al. (2008).
[b]As reported by Kerger et al. (2009).
[c]As reported by Zhang and Li (1986), number of samples not stated.
[d]Zhang and Li (1986) report this concentration as 70.5 mg/L, but Zhang and Li (1987), Beaumont et al. (2008), and Kerger et al. (2009) report a concentration of 10.5 mg/L. The total number of samples and the range in concentrations were not specified.

A mortality study was described first by Zhang and Li (1980) in an unpublished report for the JinZhou health department, and later published in a Chinese journal (Zhang and Li, 1987). Mortality records for the period 1970–1978 were obtained from local police stations for the five villages along the Nuer River, the district surrounding the ferrochromium alloy plant (TangHeZi), and three other areas to the west (YaoTangHeZi) and north (North ThangHeZi, North Nuer River) of the plant. TangHeZi and the other three areas were not affected by the groundwater chromium contamination, and these areas serve as one of the comparison groups in the analyses. Cause of death was abstracted by trained study staff and reviewed by Dr. Zhang (Kerger et al., 2009). A study interview was also conducted (with unspecified surrogates), but

the content of the interview was not described in detail (Zhang and Li, 1980). The mortality analysis indicated that the lung cancer rate was relatively high in TangHeZi (the industrial town surrounding the ferrochromium alloy plant), but decreased in areas further to the north (Zhang and Li, 1980). In the areas to the east of TangHeZi (JinChangBao, Neur River Village, ShiLiTai, YangZing, and WenJiaTun), total cancer mortality rates (71.9–92.7 per 100,000 person-years) were high relative to the region (65.4 per 100,000 person-years). Similar elevations were seen for lung cancer mortality (13.2–21.4 compared with 11.2 per 100,000 person-years in the eastern villages and comparison region, respectively, and stomach cancer mortality rates (27.7–55.2 in the eastern villages; comparison rates not given in the report, but the authors state these rates are "higher than the district as a whole") (Zhang and Li, 1987).

A subsequent paper by Zhang and Li (1997) expanded their work to include an analysis of variation in cancer rates among the five villages in the contamination zone in relation to distance from the plant and other exposure measures. This analysis is also included in the Kerger et al. (2009) report, described below.

The mortality data described in the reports by Zhang and Li (1987, 1980) are the basis for the subsequent analyses by Beaumont et al. (2008) and Kerger et al. (2009). The reanalyses by Beaumont et al. (2008) and Kerger et al. (2009) provide very similar estimates of person-years. Beaumont et al. (2008) used 1982 census data for the study areas and estimated annual growth rates from 1970 to 1982 for the Liaoning Province to estimate yearly population counts for each of the nine study areas; the summation of these figures from 1970 to 1978 represents the person-years for the study period. Kerger et al. (2009) based the population figures on the estimated populations in 1974 and multiplied these numbers by 9 (number of years of follow-up) to estimate person-years for each of the study regions. TangHeZi, the industrial area surrounding the ferrochromium alloy plant (1975 population approximately 17,500), is approximately 3–10 times bigger than the other study areas (Table 4-2).

Table 4-2. Results pertaining to cancer mortality rates in five villages along path of groundwater contamination from alloy plant and other comparison areas, western JinZhou, China from 1970 to 1978, based on analyses by Beaumont et al. (2008) and Kerger et al. (2009)

	Rate per 100,000 person-yrs					
	All cancer		Stomach cancer		Lung cancer	
Area (population or person-yrs)[a]	Age-adjusted rate	Age-adjustment influence[b]	Crude rate	Estimated age-adjusted rate[b]	Crude rate	Estimated age-adjusted rate[b]
Areas in contamination zone						
JinChanBao (2,900)	83.6	0.97	36.7	35.5	13.2	12.8
Nuer River Village (2,800)	71.9	0.98	28.0	Missing[b]	15.0	14.7
ShiLiTai (2,600)	93.0	0.94	55.2	51.7	Missing	Missing
YangXing (1,100)	76.8	0.94	36.5	34.5	21.4	20.2
WenJiaTun (1,700)	91.1	0.94	27.7	26.0	20.8	19.5
Group average (~98,700)[c]	**81.3**		**34.9**	**35.3**	**17.1**	**16.9**
Comparison areas						
TangHeZi (17,500)	71.3	0.86	16.9	14.5	21.4	18.3
North TangHeZi (3,600)	81.8	0.84	26.4[d]	22.1	8.8	7.4
North Nuer River (5,800)	71.8	1.05	30.5	31.9	7.6	8.0
Yao TangHeZi (1,500)	61.3	0.90	26.6	23.8	20.0	17.9
Group average—all (~252,500)[e]	**72.1**			**19.4**		**14.7**
Group average—without TangHeZi (96,826)[f]	**73.7**		**28.6**		**9.7**	

[a]Area population figures are based on approximate 1975 data from Beaumont et al. (2008); group values are total person-yrs for the combined area.
[b]As calculated by Beaumont et al. (2008). Nuer River Village stomach cancer rate was not included in the primary analysis by Beaumont et al. (2008) because it was missing in the original (1980) report; an additional analysis used a rate of 28 as reported by Zhang and Li (1987).
[c]Beaumont et al. (2008) estimate was 98,458 and Kerger et al. (2009) estimate was 98,850.
[d]Beaumont et al. (2008) report this value as 26.14 in Table 2, but based on the calculation of the estimated age-adjusted rate, it appears that a value close to 26.3 was used; Kerger et al. (2009) report this value as 26.4.
[e]Beaumont et al. (2008) estimate was 252,277 and Kerger et al. (2009) estimate was 253,282.
[f]As reported by Kerger et al. (2009).

The numbers of total cancer deaths, lung cancer deaths, and stomach cancer deaths were used in combination with estimated person-years at risk as the basis of the calculation of area-specific mortality rates in the analyses by Zhang and Li (1997, 1987, 1980), Beaumont et al. (2008), and Kerger et al. (2009). Because the results of Zhang and Li (1997) are repeated in the presentation by Kerger et al. (2009), only the more recent of these analyses is described in more detail below.

There are two differences between the analyses of the cancer mortality data presented by Beaumont et al. (2008) and Kerger et al. (2009). One of the minor differences is the value used for stomach cancer mortality for one of the villages in the contamination zone, Nuer River Village. Beaumont et al. (2008) do not include an estimate of stomach cancer mortality for Nuer River Village in their primary analysis because it was missing from the original (1980) unpublished report (Zhang and Li, 1980) and Dr. Zhang indicated in a faxed communication with the study authors that the estimated rate of 28 per 100,000 per year (reported in Zhang and Li, 1997) was of uncertain accuracy. Beaumont et al. (2008) did repeat their analysis using the 28 per 100,000 rate for stomach cancer mortality in Nuer River Village, and found that this inclusion had very little effect on their estimates. Kerger et al. (2009) used 28 per 100,000 per year as the stomach cancer rate for Nuer River Village. The second relatively minor difference is in the estimation of age-adjusted mortality rates. The original analyses by Zhang and Li (1987) presented age-adjusted rates for all cancer mortality, but not for stomach cancer or lung cancer mortality. Kerger et al. (2009) do not attempt to make an age adjustment for lung or stomach cancer because "small numbers of site-specific deaths in the villages would have precluded the calculation of relatable direct standardized site-specific rates in the current study." Beaumont et al. (2008) addressed this issue by calculating the ratio of unadjusted to adjusted total cancer rates for each study area, which they term the "age-adjustment influence" ratio. This ratio ranged from 0.84 to 1.05. The area-specific lung and stomach cancer unadjusted rates were multiplied by the respective area-specific age-adjustment influence ratio to create estimated age-adjusted lung and stomach cancer rates (Table 4-2).

One of the major differences between the analyses by Beaumont et al. (2008) and Kerger et al. (2009) was described previously: Kerger et al. (2009) use the 1965 exposure data for exposure-response modeling of the variation in cancer rates among the five villages in the chromium contamination zone, and Beaumont et al. (2008) do not believe that the available data are adequate for this purpose. The other major difference between the analyses is the inclusion of TangHeZi, the industrial district surrounding the ferrochromium alloy plant, in the comparison group. Kerger et al. (2009) considered this district to be too different from the smaller villages in terms of urban-rural lifestyles and other exposures that could affect cancer risk (specifically stomach cancer and lung cancer), and therefore, did not include it in their comparison group. Beaumont et al. (2008) include TangHeZi, presumably because it was part of the original study design. They do not explicitly address the comparability issue with respect to stomach cancer

risk factors, although they do note the potential for occupational chromium exposure to contribute to a relatively high lung cancer rate in TangHeZi.

Table 4-3 presents the measures of association between chromium exposure and cancer mortality, based on the five villages in the contamination zone and the various comparison groups used by Beaumont et al. (2008) and Kerger et al. (2009). These risk ratios are based on comparison of the rates shown in Table 4-2, using a Poisson distribution for calculation of 95% confidence intervals (CIs). With respect to stomach cancer, the primary site of interest from the standpoint of drinking water contamination, Beaumont et al. (2008) report an association using the four comparison areas (TangHeZi, North TangHeZi, North Nuer River, and YaoTangHeZi) that were the basis for the original analysis (risk ratio = 1.82, 95% CI 1.11–2.91) and using rates from all of Liaoning Province as a comparison (risk ratio = 1.69, 95% CI 1.12–2.44). Kerger et al. (2009) excluded the most populous area, TangHeZi from the comparison group, and reported a risk ratio = 1.22 (95% CI 0.74–2.01), which they interpret as being evidence of no association. In the lung cancer analyses, Beaumont et al. (2008) report relatively little difference between the rates in the contamination zone and the comparison area (risk ratio = 1.15, 95% CI 0.62–2.07), but a stronger association using Liaoning Province as a comparison (risk ratio = 1.78, 95% CI 1.03–2.87). Kerger et al. (2009) observed higher lung cancer rates in the five villages in the contamination zone compared with the three rural areas they included in the comparison group (risk ratio = 1.76, 95% CI 0.78–3.98), and slightly reduced risk when compared to TangHeZi (risk ratio = 0.80, 95% CI 0.44–1.47).

Table 4-3. Risk ratios comparing cancer mortality rates in five villages along a path of groundwater contamination from an alloy plant and other comparison areas in western JinZhou, China from 1970 to 1978

	All cancers		Stomach cancer		Lung cancer	
Comparison group[a]	**Risk ratio**	**95% CI**	**Risk ratio**	**95% CI**	**Risk ratio**	**95% CI**
All four areas[b]	1.13	0.86–1.46	1.82	1.11–2.91	1.15	0.62–2.07
Excluding TangHeZi[c]	1.10	0.80–1.51	1.22	0.74–2.01	1.76	0.78–3.98
Liaoning Province[b]	1.23	0.97–1.53	1.69	1.12–2.44	1.78	1.03–2.87

[a]TangHeZi, North TangHeZi, North Nuer River, and YaoTangHeZi.
[b]Reported by Beaumont et al. (2008).
[c]Reported by Kerger et al. (2009).

Kerger et al. (2009) also presented results of analyses of variation in cancer rates within the five villages in the chromium contamination zone, using three measures of exposure potential: distance from the plant, average hexavalent chromium concentrations in 1965, and percent of wells with >0.05 mg/L hexavalent chromium in 1965 (these measures can be found in Table 4-1). The analysis was based on Poisson regression of the log-transformed cancer rate in relation to the exposure measures (separate models run for each measure). For the distance

measure, a negative value for the coefficient indicates an increased cancer rate with closer proximity to the plant, and for the other exposure measures, a positive coefficient indicates an increased cancer rate with higher exposure. The results for all cancer mortality (given as the regression coefficient and *p*-value) were 0.04 ($p = 0.61$), -0.07 ($p = 0.54$), and -0.24 ($p = 0.45$) for the distance, average hexavalent chromium concentration in 1965, and percent of wells >0.05 mg/L hexavalent chromium in 1965 measures, respectively. For stomach cancer mortality, the coefficients were 0.01 ($p = 0.93$), -0.11 ($p = 0.50$), and -0.32 ($p = 0.51$) for the distance, average hexavalent chromium concentration in 1965, and percent of wells >0.05 mg/L hexavalent chromium in 1965 measures, respectively, and for lung cancer, the coefficients were 0.12 ($p = 0.50$), -0.06 ($p = 0.79$), and -0.11 ($p = 0.88$) for the distance, average hexavalent chromium concentration in 1965, and percent of wells >0.05 mg/L hexavalent chromium in 1965 measures, respectively. As described previously, Beaumont et al. (2008) did not include this type of exposure-response analysis because they believed that the inherent limitations of the exposure data precluded a meaningful analysis.

In addition to the cancer mortality study, the JinZhou health department also collected data pertaining to symptoms in 1965 in Nuer River Village, which was one of the highly contaminated areas at that time (well water hexavalent chromium levels of 0.1–20.0 mg/L) (Zhang and Li, 1987, 1986). Among 156 residents surveyed, 51 (33%) had oral ulcers, 20 (17%) had diarrhea, 48 (31%) had abdominal pain, 26 (17%) had dyspepsia, 81 (30%) had stomach pain, and 20 (17%) had vomiting (JinZhou Antiepidemic Station, 1979). The authors state that "no such symptoms were found among the residents whose water wells were not contaminated." A similar study of 158 people in ShiLiTai in 1971 found a similar pattern of symptoms, with 92 (58%) reporting oral ulcers, 48 (30%) diarrhea, and 36 (23%) abdominal pain. In 1974, another study of children in WenJiaTun and Sandaohao, at the eastern edge of the contamination zone, also found similar symptoms (data not shown in the 1979 report). The authors speculate that the symptoms may have been due to the increased concentrations of sulfates (>300 mg/L) in the drinking water in these areas in 1974, rather than the relatively low concentrations of hexavalent chromium (0.003–0.05 mg/L).

Zhang and Li (1987, 1986) also conducted hematological assessments of 12 individuals in 1965, and another study of 93 individuals (time not specified). The exact location of the participants was not specified, but they were said to be from "highly polluted" or "high density contamination" areas. White blood cell counts were elevated in the first study, and the number of neutrophilic granulocytes and what was termed "juvenile cells" among these granulocytes was elevated in the second study.

Kings County/San Bernardino County, California (Fryzek et al., 2001)

A study of areas in Kings County and San Bernardino County, California, compared cancer mortality in locations near natural gas compressor plants with areas not located near the

plants (Fryzek et al., 2001). Hexavalent chromium compounds had been used as anti-corrosion additives in cooling tower water at the gas plants during the period 1950 to approximately 1980. Waste material was released to surface ponds and was subject to percolation to groundwater. Cooling tower water was also aerosolized and transported to the ground surface where it may have contacted soil, crops, and surface water. Thus, exposures to hexavalent chromium may have occurred by several routes (i.e., inhalation, ingestion, and dermal contact). Mortality records for zip codes for the cities of Kettleman City (in Kings County), and Hinkley and Topock (in San Bernadino County), in which natural gas compressor plants were located, were compared to records from zip codes in Kings County and San Bernadino County, other than those encompassing these three cities. The study included mortality records for the period 1989–1998, during which time 2,226,214 deaths were recorded. Age-adjusted cancer mortality rate ratios (rate in areas near the plant/rate in comparison areas) were 1.03 (95% CI 0.90–1.17) for lung cancer death, 0.93 (95% CI 0.87–1.00) for all cancer deaths, and 0.98 (95% CI 0.95–1.02) for all deaths. Rate ratios for stomach cancer were not reported. This study found no significant difference between mortality or cancer mortality among residents from zip codes in which gas plants that used hexavalent chromium additives in cooling tower water were located compared to residents of other nearby areas without such plants. An important limitation of this study is that exposure assignment was based on zip code, rather than on individual-level data, which is likely to result in significant exposure misclassification.

Nebraska (Bednar and Kies, 1991)

Bednar and Kies (1991) compared levels of chromium (and other chemicals) in drinking water in Nebraska counties with death rates in these same areas. Data on chromium in drinking water were obtained for each of 453 communities (all incorporated communities of Nebraska) for the period 1986–1987, and mortality data for each Nebraska county was obtained for the year 1986 (both compiled by the Nebraska Department of Health). Mean total chromium concentration in drinking water for the 453 communities was 0.002 mg chromium/L (range <0.001–0.01 mg chromium/L); the study report did not indicate valence state of chromium detected in these drinking water samples. Possible associations between chromium exposure and health outcomes were assessed by linear correlation (Pearson) of mortality rates (at the county level) and chromium concentrations in drinking water (presumably aggregated from community data to represent counties). Correlations were reportedly explored for mortality from cancer, cerebrovascular disease, heart disease, pneumonia, and chronic lung disease; however, only one chromium correlation coefficient was reported to be statistically significant, that for death from chronic lung disease, and the correlation was negative (-0.101, $p = 0.03$). As with the other studies of this design, a major limitation is that exposures to chromium cannot be estimated for individual subjects in the study and may not be accurately represented by the drinking water chromium measurements. For example, the 1986–1987 drinking water data do not necessarily

represent long-term exposure patterns, and an individual represented in a county death record does not necessarily mean that the individual resided in the county for their lifetime or any significant fraction of their lifetime.

Glasgow, United Kingdom (Eizaguirre-Garcia et al., 2000, 1999)

Eizaguirre-Garcia et al. (2000, 1999) examined risk of leukemia and birth defects in people residing near the site of a former chromium processing facility in Glasgow, United Kingdom. The factory was in operation for >100 years and ceased operations in 1967. A survey conducted in 1991 found average soil concentrations at the site of operations of 8,164 mg/kg for total chromium and 848 mg/kg for hexavalent chromium. Soil concentrations of total chromium and hexavalent chromium approximately 2–3 km from the factory site were reported as "approximately half" of those at the site; no additional information on soil levels off-site were reported (Eizaguirre-Garcia et al., 2000, 1999). Reported cases of leukemia for the period 1975–1989 were obtained from the Scottish Cancer Registration, during which 1,205 cases of leukemia were reported in a population of 873,643 (Eizaguirre-Garcia et al., 1999). Leukemia cases were aggregated at the level of Enumeration Districts (EDs) (approximately 350–500 individuals per district). When stratified by distance of the EDs from the plant (out to 9–10 km), relative risks of leukemia (0–2 km as reference) were unrelated to distance. When other influential variables were included in a Poisson regression model (gender, socioeconomic status, and age) in addition to distance of EDs from the plant (0–4, 4–9, 9–10 km), relative risk was significant (1.29, 95% CI 1.07–1.56) for EDs 4–9 km from the plant (relative to 0–4 km), but not for EDs 9–10 km from the plant. These results suggest that leukemia risk increased with distance from the plant (i.e., 4–9 > 0–4 km) and then declined with further distance (i.e., 9–10 = 0–4 km). This pattern does not strongly implicate the plant as a major contributor to leukemia risk.

A similar study of risk of birth defects was conducted on the same population (Eizaguirre-Garcia et al., 2000). In this study, data on number of births and congenital malformations were collected for the period 1982–1989. Case definitions (not reported) followed those of the European-wide EURCAT network (http://www.eurocat.ulster.ac.uk/). The study included 2,778 cases from a population of 81,057 births; cases were aggregated at the level of EDs. When distance from the plant (0–1, 2–4, 4–10 km) and socioeconomic status were included in a Poisson regression model, relative risk was significant for the EDs in the 2–4 km category (1.47, 95% CI 1.2–1.7) and the 4–10 km category (1.25, 95% CI 1.05–1.49); however, both distance categories were associated with higher risks than the closest distance category, 0–1 km. Similar to the results for leukemia, this pattern does not strongly implicate the plant as a major contributor to risk of congenital anomalies. Not taken into consideration in this study were several other potentially influential variables on developmental outcomes; for example, maternal age and health, smoking, and alcohol consumption.

Summary

The Liaoning Province studies provide the most detailed analysis of all of the epidemiological studies that have been conducted with respect to chromium and cancer mortality (specifically stomach cancer or other cancers of the digestive system). These studies are important in that they examined a population exposed to very high levels of chromium in drinking water wells (i.e., sufficient to impart a visible yellow color to the water). Sources of exposure include the drinking water, food grown in contaminated soil, and possibly air. Levels up to 20 mg/L in well water were documented in the first surveys done in 1965 in the two villages closest to the source of exposure (a ferrochromium alloy plant). The contamination began sometime between 1959 and 1964; the reporting of a yellowing of the water by local residents in 1964 is what led to the investigation and identification of this contamination by the local health department.

The interpretation of the mortality data originally collected by Zhang and Li (1980) depends in large part on the choice of referent group. That choice depends on many factors, including the perceived comparability and the size of the populations. Larger populations, such as a province or state, have the advantage of providing relatively stable estimates, particularly for low-incident events such as site-specific cancers. Smaller areas (e.g., a neighboring community) offer the advantage of potentially greater similarities in ethnic background, socioeconomic status, and occupational and lifestyle factors that may affect cancer risk. However, small comparison groups are likely to produce imprecise estimates, and the issue of over-controlling may arise, for example, if the comparison population shares the specific exposure of interest (for example, with the selection of friends or co-workers in case-control studies). The associations presented by Beaumont et al. (2008) using Liaoning Province as the comparison group provide evidence of an excess risk in the villages in the contamination zone of mortality from stomach cancer (rate ratio 1.69, 95% CI 1.12–2.44) and lung cancer (rate ratio 1.78, 95% CI 1.03–2.87), with a small increase also suggested in total cancer mortality (rate ratio 1.23, 95% CI 0.97–1.53). The association with stomach cancer mortality is also seen when using the four adjacent areas as the referent group (rate ratio 1.82, 95% CI 1.11–2.91), but is weaker when the industrial area surrounding the plant, TangZeHi, is removed from the comparison group (rate ratio 1.22, 95% CI 0.74–2.01). Kerger et al. (2009) believe that the relatively urban environment of TangHeZi makes it an inappropriate comparison group for the villages in the contamination zone. With respect to stomach cancer, historical trends show clear decreases in the incidence of this cancer in a variety of geographical areas, with improvements that come with economic development and urbanization (e.g., sanitation, refrigeration) contributing to this decline. An analysis of gastric cancer rates in China in 1990–1992 showed lower mortality rates in urban areas (15.3 per 100,000) compared with rural areas (24.4 per 100,000) (Yang, 2006). However, this same study reported little difference between urban and rural rates in 1973–1975 (20.1 and 19.4 per 100,000 in urban and rural areas, respectively), the relevant time period with respect to the

Liaoning Province studies. Thus, EPA does not consider the exclusion of TangZeHi from the comparison group to be warranted.

Another issue regarding the interpretation of the mortality data is the validity of analyses of the variability in cancer rates among the five villages in the contamination zone in relation to the available exposure measures (distance from the plant, average concentration in wells in 1965, and percent of wells with hexavalent chromium levels >0.05 mg/L in 1965). There are considerable limitations to these measures, including the lack of individual-level data on use of water from specific wells over time and the changes in exposure due to efforts to treat the water in the most contaminated areas with treatment wells built in 1967. Based on these limitations, EPA concluded that the exposure-response analyses presented by Zhang and Li (1997) and Kerger et al. (2009) are not based on the quality of data that is needed to support a conclusion regarding the presence or absence of a dose-response among the observed cancer rates in these villages.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL

The effects of subchronic oral exposure to hexavalent chromium have been evaluated in rats (NTP, 2007; Quinteros et al., 2007; Rafael et al., 2007; Acharya et al., 2001; Chopra et al., 1996; Vyskocil et al., 1993) and mice (NTP, 2007; Asmatullah and Noreen 1999), and the effects of chronic oral exposure to hexavalent chromium have been evaluated in rats (NTP, 2008; MacKenzie et al., 1958), mice (NTP, 2008; Borneff et al., 1968), and dogs (Anwar et al., 1961). The studies conducted by the National Toxicology Program (NTP, 2008, 2007) provide dose-response data on the effects of oral hexavalent chromium exposure based on a comprehensive assessment of toxicological endpoints. Effects associated with oral exposure to hexavalent chromium as reported in the NTP (2007) subchronic study included hematological effects, hepatotoxicity, alterations in lipid metabolism, and histopathological changes in GI tissues and pancreatic and mesenteric lymph nodes. The most sensitive hexavalent chromium-induced effects were microcytic, hypochromic anemia, increased serum liver enzyme activities, and histopathological changes to the duodenum and pancreatic lymph nodes in rats; and histopathological changes in the duodenum in mice. The most sensitive noncancer effects in the NTP (2008) 2-year toxicology and carcinogenicity study were nonneoplastic histopathological changes to the liver, duodenum, and mesenteric lymph nodes in rats and the duodenum, mesenteric lymph nodes, and liver in mice. In addition, based on findings of squamous cell neoplasms of the oral cavity in rats and neoplasms of the small intestine in mice, NTP (2008) concluded that results of this study provide clear evidence of carcinogenic activity of sodium dichromate dihydrate.

Several other oral exposure studies (i.e., Quinteros et al., 2007; Rafael et al., 2007; Asmatullah and Noreen, 1999; Vyskocil et al., 1993; Anwar et al., 1961) do not provide suitable

data for identifying NOAELs or LOAELs because comprehensive toxicological endpoints were not evaluated in these studies. LOAELs identified by EPA in studies by Acharya et al. (2001) and Chopra et al. (1996) were based on evaluation of a limited number of liver endpoints. In addition, interpretation of results from these studies was limited due to the small number of animals evaluated, lack of dose-response data, or inadequate reporting for estimation of doses in mg hexavalent chromium/kg-day. However, results of these studies are useful for identification of potential adverse effects of oral hexavalent chromium exposure. Subchronic and chronic studies are summarized in Section 4.5, Synthesis of Major Noncancer Effects—Oral, Table 4-26.

4.2.1. Subchronic Oral Exposure

NTP, 2007

NTP (2007) conducted a 3-month toxicology study of sodium dichromate dihydrate in drinking water in rats and mice. This study was divided into three separate studies evaluating effects of treatment in: (1) male and female F344/N rats, (2) male and female B6C3F_1 mice, and (3) three strains of male mice (B6C3F_1, BALB/c, and *am3*-C57BL/6). In the 3-month study in F344/N rats, groups of 10 males and 10 females ("core" study animals) were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 62.5, 125, 250, 500, or 1,000 mg sodium dichromate dihydrate/L (equivalent to 0, 21.8, 43.6, 87.2, 174.5, or 348 mg hexavalent chromium/L, respectively) for 3 months. Based on water consumption monitored throughout the study, NTP (2007) calculated average daily doses over the 3-month treatment duration of approximately 0, 5, 10, 17, 32, or 60 mg sodium dichromate dihydrate/kg-day (equivalent to 0, 1.7, 3.5, 5.9, 11.2, or 20.9 mg hexavalent chromium/kg-day, respectively) for both males and females. An additional 10 male and 10 female rats ("clinical pathology" animals) were exposed to the same concentrations of sodium dichromate dihydrate for 4 weeks. "Core" study animals were observed twice daily for mortality and clinical signs of toxicity; water consumption and body weights were recorded weekly. Blood was collected from "clinical pathology" animals on treatment days 5 and 23 and from "core" study animals at study termination for comprehensive hematology and clinical chemistry endpoints. Urine was collected from "clinical pathology" animals on day 16 and analyzed for comprehensive urinalytic endpoints. At study termination, necropsies were performed on all "core" study animals, with organ weights recorded for heart, right kidney, liver, lung, spleen, right testis, and thymus. Microscopic examinations of comprehensive tissues were conducted in all "core" study animals in the control and 20.9 mg hexavalent chromium/kg-day (high-dose) groups and on six "core" study animals from each of the other treatment groups. In addition, all tissues identified as target organs in the 20.9 mg hexavalent chromium/kg-day (high-dose) group were examined in lower dose groups until a no-effect level was identified or all animals were examined.

No mortalities were observed in male or female rats exposed to sodium dichromate dihydrate in drinking water for 3 months (NTP, 2007). Final body weights in male rats were

significantly decreased by 5 and 11% in the 11.2 and 20.9 mg hexavalent chromium/kg-day groups, respectively, compared to controls. In females, final body weight was significantly decreased by 9% in the 20.9 mg hexavalent chromium/kg-day group compared to controls. In males and females in the ≥5.9 mg hexavalent chromium/kg-day groups, water consumption was decreased (statistical significance not reported). Data on food consumption were not reported. No treatment-related signs of clinical toxicity were observed throughout the study.

Results of hematology analyses show that exposure of male and female rats to sodium dichromate dihydrate in drinking water produced microcytic, hypochromic anemia, characterized by decreases in mean cell volume (MCV), hematocrit (Hct), hemoglobin (Hgb), and mean cell hemoglobin (MCH) (NTP, 2007). In general, the severity of this anemia exhibited a dose response, with the more pronounced effects observed at the 23-day time point versus the 3-month time point (Table 4-4). After 5 days of exposure, small changes were observed in several hematological parameters; however, decreases in all treatment groups were ≤5% compared to controls. More severe, dose-related effects were observed after 23 days of treatment, with changes observed in all treatment groups in males and females. Similar effects were observed after 3 months of treatment, although severity at 3 months was generally less than that observed at 23 days, indicating a compensatory hematopoietic response. Some of the hematological parameters, e.g., hematocrit and erythrocyte counts of male rats treated for 3 months, although decreased significantly, were within normal ranges. In female rats, the direction of the changes in mean cell hemoglobin and erythrocyte count at 23 days differed from those at 3 months. Blood smears showed evidence of erythrocyte injury or increased turnover, including erythrocyte fragments, keratocytes, and blebbing (incidence data not reported). Increased reticulocyte counts and nucleated erythrocytes, indicative of a compensatory hematopoietic response, were also observed in both sexes at 23 days and 3 months; however, these increases did not exhibit a consistent pattern of dose- or duration-dependence. Dose-dependent increases in platelet counts occurred at 23 days in all treatment groups compared to controls; however, severity was decreased at 3 months (Table 4-4). NTP (2007) stated that increased platelet counts are consistent with compensatory hematopoiesis or an iron deficiency. Increased neutrophil and monocyte counts were observed at higher doses (≥5.9 and ≥3.5 mg hexavalent chromium/kg-day in males and females, respectively) and were considered by NTP (2007) to reflect an inflammatory response related to the inflammatory gastric lesions. Results of hematological analyses showed that exposure of rats to sodium dichromate dihydrate in drinking water at daily doses ≥1.7 mg hexavalent chromium/kg-day produced microcytic, hypochromic anemia, but that severity decreased slightly as exposure duration increased from 23 days to 3 months.

Table 4-4. Hematological effects in male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for up to 3 months

Hematological parameter	Time on treatment	Treatment group (mg hexavalent chromium/kg-d)					
		0	1.7	3.5	5.9	11.2	20.9
Males							
Hct (%)	23 D	48.0 ± 0.5[a]	44.7 ± 0.7[b] (93.1)	39.8 ± 0.8[b] (82.9)	36.2 ± 1.0[b] (75.4)	34.4 ± 0.5[b] (71.7)	32.3 ± 1.1[b] (67.3)
	3 Mo	45.7 ± 0.2	45.2 ± 0.4 (98.9)	45.2 ± 0.3 (98.9)	44.8 ± 0.7 (98.0)	42.9 ± 0.4[b] (93.9)	36.9 ± 0.8[b] (80.7)
Hgb (g/dL)	23 D	15.9 ± 0.1	14.2 ± 0.2[b] (89.3)	12.0 ± 0.3[b] (75.5)	10.9 ± 0.3[b] (68.6)	10.3 ± 0.3[b] (64.8)	9.2 ± 0.3[b] (57.9)
	3 Mo	15.3 ± 0.1	15.2 ± 0.1 (99.3)	15.0 ± 0.1 (98.0)	14.4 ± 0.2[b] (94.1)	13.3 ± 0.2[b] (86.9)	10.9 ± 0.3[b] (71.2)
MCV (fL)	23 D	61.1 ± 0.5	53.6 ± 0.6[b] (87.7)	48.0 ± 0.4[b] (78.6)	46.4 ± 0.6[b] (75.9)	46.2 ± 0.3[b] (75.6)	46.4 ± 0.5[b] (75.9)
	3 Mo	51.8 ± 0.1	50.3 ± 0.2[b] (97.1)	49.0 ± 0.1[b] (94.6)	44.4 ± 1.0[b] (85.7)	39.7 ± 0.5[b] (76.6)	36.0 ± 0.4[b] (69.5)
MCH (ρg)	23 D	20.1 ± 0.2	16.9 ± 0.2[b] (84.0)	17.2 ± 0.7[b] (85.6)	18.2 ± 0.4 (90.5)	19.7 ± 0.3 (98.1)	20.7 ± 0.6 (103.0)
	3 Mo	17.3 ± 0.1	16.9 ± 0.1[b] (97.7)	16.2 ± 0.1[b] (93.6)	14.2 ± 0.4[b] (82.1)	12.3 ± 0.2[b] (71.1)	13.0 ± 0.5[b] (75.1)
Erythrocyte count (10^6/µL)	23 D	7.94 ± 0.10	8.38 ± 0.11 (105.5)	7.13 ± 0.35[c] (89.8)	6.0 ± 0.28[b] (75.6)	5.25 ± 0.19[b] (66.1)	4.54 ± 0.33[b] (57.2)
	3 Mo	8.88 ± 0.05	9.04 ± 0.09[c] (101.8)	9.25 ± 0.07[b] (104.2)	10.15 ± 0.22[b] (114.3)	10.87 ± 0.07[b] (122.4)	8.52 ± 0.45[b] (95.9)
Platelet count (10^6/µL)	23 D	745.2 ± 22.2	1,065.3 ± 67.9[b] (143)	2,768.6 ± 328.5[b] (372)	3,504.7 ± 235.0[b] (470)	4,226.0 ± 204.5[b] (567)	4,688.8 ± 242.7[b] (629)
	3 Mo	618.6 ± 20.0	736.1 ± 11.5 (119)	604.3 ± 24.5 (98)	909.8 ± 119.1[b] (147)	1,743.1 ± 178.0[b] (282)	5,123.0 ± 638.9[b] (828)
Females							
Hct (%)	23 D	48.0 ± 0.4[a]	46.6 ± 0.9 (97.1)	42.9 ± 0.8[b] (89.4)	39.2 ± 0.7[b] (81.7)	37.2 ± 0.7[b] (79.6)	33.4 ± 0.6[b] (69.6)
	3 Mo	44.6 ± 0.4	45.2 ± 0.1 (101.3)	44.1 ± 0.3 (98.9)	42.9 ± 0.2[b] (96.2)	42.6 ± 0.5[b] (95.5)	38.3 ± 0.5[b] (85.9)
Hgb (g/dL)	23 D	15.9 ± 0.1	14.7 ± 0.3[b] (92.5)	13.0 ± 0.3[b] (81.8)	11.8 ± 0.3[b] (74.2)	10.9 ± 0.2[b] (68.6)	9.7 ± 0.2[b] (61.0)
	3 Mo	15.2 ± 0.1	15.4 ± 0.1 (101.3)	14.9 ± 0.1 (98.0)	14.3 ± 0.1[b] (94.1)	14.1 ± 0.2[b] (92.8)	12.0 ± 0.2[b] (78.9)
MCV (fL)	23 D	61.1 ± 0.4	53.9 ± 0.5[b] (88.2)	48.8 ± 0.5[b] (79.9)	46.6 ± 0.6[b] (76.3)	45.7 ± 0.4[b] (74.8)	46.5 ± 0.5[b] (76.1)
	3 Mo	53.3 ± 0.1	53.3 ± 0.1 (100)	52.4 ± 0.2[b] (98.3)	50.5 ± 0.3[b] (94.7)	48.0 ± 0.9[b] (90.1)	40.0 ± 0.7[b] (75.0)
MCH (ρg)	23 D	20.4 ± 0.1	17.3 ± 0.2 (84.8)	18.0 ± 0.3 (88.2)	18.9 ± 0.7 (92.6)	21.0 ± 0.6 (102.9)	23.1 ± 0.5 (113.2)
	3 Mo	18.4 ± 0.1	17.9 ± 0.1[b] (97.3)	17.8 ± 0.1[b] (96.7)	16.9 ± 0.1[b] (91.8)	15.9 ± 0.4[b] (86.4)	12.5 ± 0.3[b] (67.9)

Table 4-4. Hematological effects in male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for up to 3 months

Hematological parameter	Time on treatment	Treatment group (mg hexavalent chromium/kg-d)					
		0	1.7	3.5	5.9	11.2	20.9
Erythrocyte count (10^6/µL)	23 D	7.82 ± 0.09	8.52 ± 0.14 (109.0)	7.22 ± 0.19 (92.3)	6.32 ± 0.36[b] (80.8)	5.27 ± 0.23[b] (67.4)	4.21 ± 0.16[c] (53.8)
	3 Mo	8.30 ± 0.06	8.60 ± 0.05[b] (103.6)	8.40 ± 0.04[c] (101.2)	8.47 ± 0.04[c] (102.0)	8.93 ± 0.11[b] (107.6)	9.62 ± 0.10[b] (115.9)
Platelet count (10^6/µL)	23 D	611.5 ± 43.7	1,156.3 ± 76.4[b] (189)	2,808.8 ± 198.5[b] (459)	3,295.0 ± 349.7[b] (539)	4,318.4 ± 234.9[b] (706)	5,132.8 ± 247.0[b] (839)
	3 Mo	588.9 ± 17.1	605.8 ± 17.1 (103)	574.8 ± 21.3 (98)	528.2 ± 14.1 (90)	619.3 ± 55.4 (105)	1,524.9 ± 193.3[b] (259)

[a]Values are means ± SE; values in parenthesis are percent of control; n = 10 rats/group, with the following exceptions: 1.7 mg hexavalent chromium/kg-d group females on d 23 and mo 3 (n = 9), 3.5 mg hexavalent chromium/kg-d group females on d 23 (n = 8), 5.9 mg hexavalent chromium/kg-d group females on d 23 (n = 9), and 20.9 mg hexavalent chromium/kg-d group females on d 23 and mo 3 (n = 9).
[b]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.
[c]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.

Source: NTP (2007).

Results of clinical chemistry analyses in male and female rats exposed to sodium dichromate dihydrate in drinking water showed treatment-related increases in serum liver enzyme activities, bile acids, and serum creatine kinase activity and alterations in lipid metabolism (Table 4-5) (NTP, 2007). Serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities were significantly increased compared to controls in all treatment groups at 3 months, with less severe effects seen at 23 days. A consistent relationship between severity and dose was not observed. In male rats, elevations of ALT and SDH activities increased with increasing dose between 1.7 and 11.2 mg/kg-day, but less severe elevations were observed at 20.9 mg/kg-day (Table 4-5). In females, increases in ALT and SDH activities were generally indicative of a uniform effect across the dose range (Table 4-5). NTP (2007) suggested that increases are consistent with hepatocellular injury or membrane leakage. At 3 months, bile acids were significantly increased compared to controls at ≥11.2 mg hexavalent chromium/kg-day in males and in all treatment groups (except 5.9 mg hexavalent chromium/kg-day) in females; similar to serum liver enzymes, increases in bile acids were not consistently related to dose. NTP (2007) suggested that increased bile acid was indicative of hepatic toxicity rather than colestasis, as other markers of colestasis (e.g., alkaline phosphatase [AP] and 5N-nucleotidase) were not affected by treatment. At 3 months, decreased serum cholesterol and triglycerides, indicative of altered lipid metabolism, were observed; however, a consistent relationship between severity and dose was not observed. At 3 months, dose-related increases in serum creatine kinase activity, indicative of muscle damage, were observed in males and females

at ≥5.9 mg hexavalent chromium/kg-day. Urinalysis showed dose-related decreased volume and increased specific gravity, consistent with decreased water intake. NTP (2007) suggested that decreased water intake was due to decreased palatability. Other changes in clinical chemistry and urinalysis parameters were transient, with no apparent relationship to treatment. Results of clinical chemistry analyses indicated that exposure of rats to sodium dichromate dihydrate in drinking water induced hepatocellular membrane damage or cytotoxicity (both sexes) and increased bile acids (females) at doses ≥1.7 mg hexavalent chromium/kg-day (both sexes).

Table 4-5. Clinical chemistry effects in male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 3 months

Clinical chemistry parameter	Time on treatment	Treatment group (mg hexavalent chromium/kg-d)					
		0	1.7	3.5	5.9	11.2	20.9
Males							
ALT (IU/L)	3 Mo	98 ± 6[a]	274 ± 30[c] (280)	461 ± 102[c] (470)	447 ± 121[c] (456)	740 ± 81[c] (755)	191 ± 17[c] (195)
SDH (IU/L)	3 Mo	31 ± 2	55 ± 5[c] (177)	110 ± 24[c] (355)	102 ± 24[c] (329)	173 ± 20[c] (558)	59 ± 6[c] (190)
Bile acids (µmol/L)	3 Mo	22.0 ± 2.2	24.0 ± 3.4 (109)	34.5 ± 7.0 (157)	32.6 ± 5.3 (148)	45.3 ± 2.8[c] (206)	28.1 ± 2.0[c] (128)
Cholesterol (mg/dL)	3 Mo	89 ± 2	95 ± 2 (107)	86 ± 4 (97)	65 ± 2[c] (73)	86 ± 3[b] (97)	71 ± 2[c] (80)
Triglycerides (mg/dL)	3 Mo	170 ± 9	169 ± 8 (99)	172 ± 15 (101)	170 ± 13 (100)	164 ± 12 (96)	98 ± 8[c] (57)
Creatine kinase (IU/L)	3 Mo	214 ± 26	286 ± 32 (134)	291 ± 36 (136)	364 ± 23[c] (170)	413 ± 16[c] (193)	374 ± 44[c] (175)
Females							
ALT (IU/L)	3 Mo	64 ± 5[a]	437 ± 68[c] (683)	218 ± 27[c] (340)	245 ± 30[c] (383)	246 ± 37[c] (384)	248 ± 22[c] (387)
SDH (IU/L)	3 Mo	22 ± 2	101 ± 17[c] (459)	65 ± 10[c] (295)	81 ± 13[c] (368)	96 ± 20[c] (436)	103 ± 12[c] (468)
Bile acids (µmol/L)	3 Mo	19.7 ± 2.5	50.4 ± 6.0[c] (256)	39.9 ± 4.3[c] (203)	35.3 ± 3.5 (179)	45.3 ± 5.6[c] (230)	38.7 ± 3.2[b] (196)
Cholesterol (mg/dL)	3 Mo	95 ± 2	111 ± 4 (117)	94 ± 2 (99)	87 ± 2 (92)	83 ± 2[b] (87)	79 ± 2[c] (83)
Triglycerides (mg/dL)	3 Mo	139 ± 18	116 ± 10 (93)	98 ± 9 (70)	81 ± 4[c] (58)	76 ± 7[c] (55)	59 ± 6[c] (42)
Creatine kinase (IU/L)	3 Mo	197 ± 23	311 ± 94 (158)	265 ± 23 (135)	296 ± 24[c] (150)	359 ± 23[c] (182)	432 ± 48[c] (219)

[a]Values are means ± SE; values in parenthesis are percent of control; n = 10 rats/group, with the following exceptions: control group males (n = 9), 1.7 mg hexavalent chromium/kg-d group females (n = 9), and 20.9 mg hexavalent chromium/kg-d group females (n = 9).
[b]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.
[c]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.

Source: NTP (2007).

Changes in organ weights in rats exposed to sodium dichromate dihydrate in drinking water for 3 months are summarized in Table 4-6 (NTP, 2007). Treatment-related effects were generally observed at doses ≥1 1.2 mg hexavalent chromium/kg-day. In males, decreases were observed in absolute and relative liver weights and in absolute and relative spleen weights; in females, relative right kidney weights and relative spleen weights were increased. Changes in weights of other organs were considered by NTP (2007) to be secondary to changes in body weight rather than due to adverse effects of treatment.

Table 4-6. Selected organ weights in male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 3 months

Organ	Treatment group (mg hexavalent chromium/kg-d)					
	0	1.7	3.5	5.9	11.2	20.9
Males						
Liver, absolute weight	10.89 ± 0.42[a]	10.30 ± 0.28	11.45 ± 0.38	10.51 ± 0.18	9.20 ± 0.17[b]	8.88 ± 0.18[b]
Liver, relative weight[c]	32.91 ± 0.65	31.91 ± 0.61	33.98 ± 0.75	31.90 ± 0.54	29.15 ± 0.53[d]	29.80 ± 0.35[b]
Spleen, absolute weight	0.64 ± 0.02	0.60 ± 0.01	0.62 ± 0.02	0.60 ± 0.02	0.53 ± 0.01[b]	0.60 ± 0.01[b]
Spleen, relative weight[c]	1.94 ± 0.03	1.85 ± 0.03	1.83 ± 0.04	1.81 ± 0.05[d]	1.69 ± 0.02[b]	2.00 ± 0.03
Females						
Right kidney, relative weight	3.34 ± 0.09[a]	3.32 ± 0.04	3.55 ± 0.05	3.55 ± 0.07	3.58 ± 0.10[d]	3.63 ± 0.09[d]
Spleen, relative weight[c]	2.12 ± 0.05	2.04 ± 0.03	2.16 ± 0.05	2.22 ± 0.03	2.25 ± 0.05[d]	2.39 ± 0.03[d]

[a]Values are means ± SE; n = 10 rats/group.
[b]Significantly different ($p \leq 0.01$) from the control group by Williams's or Dunnett's test.
[c]Relative weight = mg organ weight/g body weight.
[d]Significantly different ($p \leq 0.05$) from the control group by Williams's or Dunnett's test.

Source: NTP (2007).

Gross and microscopic examinations of male and female rats exposed to sodium dichromate dihydrate in drinking water for 3 months showed nonneoplastic lesions of the duodenum, glandular stomach, pancreatic lymph nodes, liver (females only), and bone marrow (females only) (NTP, 2007); incidence data are summarized in Table 4-7. The incidence of minimal-to-mild duodenal histiocytic cellular infiltration was increased in males and females at ≥3.5 and ≥1.7 mg hexavalent chromium/kg-day, respectively, compared to controls; incidence increased with dose. Histiocytic cellular inflammation appeared as multifocal, randomly scattered, small clusters of enlarged macrophages with pale foamy cytoplasm. Incidences of nonneoplastic lesions of the glandular stomach (ulcer, focal regenerative hyperplasia, and focal squamous hyperplasia, all mild-to-moderate in severity) were increased in rats in the highest dose group. Microscopically, ulcers were characterized by complete loss of the lining of the mucosal epithelium with necrosis, often extending through to the submucosa, and muscle layers; mild to marked chronic inflammation (infiltrates of neutrophils, macrophages, lymphocytes, and

eosinophils); and proliferation of fibrous connective tissue through the submucosa. Lesions were not observed in the forestomach. Microscopic examinations of the oral mucosa and tongue in a retrospective analysis conducted by NTP revealed nonneoplastic lesions in 4 rats that were not considered by NTP to be treatment-related. In males, significant increases in the incidence of minimal histiocytic cellular infiltration of pancreatic lymph nodes were observed at 1.7, 5.9, 11.2, and 20.9 mg hexavalent chromium/kg-day, without a clear dose-response relationship, whereas significantly increased incidences and severity of pancreatic lymph node sinusoidal ectasia and lymphoid hyperplasia were only seen at the highest dose; in females, significant increases in the incidences and severity of nonneoplastic lesions of pancreatic lymph nodes were only observed at the highest dose. Microscopically, lymphoid hyperplasia was characterized by minimal-to-mild lymphocyte proliferation, and sinusoid ectasia was characterized by minimal-to-mild dilatation of the subcapsular or medullary sinuses; histiocytic cellular infiltration was similar to that observed in the duodenum. Minimal-to-mild histiocytic cellular infiltration was observed in all groups including control animals. The increase in the incidences in the lower dose groups was not statistically significant. In the liver of females, a dose-dependent increase in the incidence of histiocytic cellular infiltration was observed at $\geq$3.5 mg hexavalent chromium/kg-day. Minimal chronic inflammation in female liver was observed in three of the control animals and in a few of the animals at doses up to 11.2 mg/kg-day. The incidence of chronic inflammation in female rats was not dose dependent; however, the incidence was increased significantly in the highest dose group. Although serum liver enzymes were statistically significantly increased in all treatment groups (discussed above), significant histopathological changes to the livers of male rats were not observed. The incidence of bone marrow hyperplasia was significantly increased in high-dose females. This observation is consistent with an increase in hematopoiesis in response to hexavalent chromium-induced microcytic, hypochromic anemia.

Table 4-7. Incidence of nonneoplastic lesions observed in male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 3 months

Tissue (lesion type)	Treatment group (mg hexavalent chromium/kg-d)					
	0	1.7	3.5	5.9	11.2	20.9
Males						
Duodenum (histiocytic cellular infiltration)	0/10[a]	0/10	7/10[b] (1.1)	9/10[b] (1.2)	8/10[b] (1.4)	7/10[b] (1.4)
Stomach, glandular (ulcer)	0/10	0/10	0/10	0/10	0/10	8/10[b] (3.0)
Stomach, glandular (focal regenerative hyperplasia)	0/10	0/10	0/10	0/10	0/10	10/10[b] (2.2)
Stomach, glandular (focal squamous hyperplasia)	0/10	0/10	0/10	0/10	1/10 (2.0)	7/10[b] (2.6)
Pancreatic lymph node (ectasia)	0/10	0/10	0/10	0/10	1/10 (1.0)	10/10[b] (1.7)
Pancreatic lymph node (lymphoid hyperplasia)	0/10	0/10	0/10	3/10 (1.0)	3/10 (1.0)	6/10[b] (2.7)
Pancreatic lymph node (histiocytic cellular infiltration)	0/10	5/10[c] (1.0)	2/10 (1.0)	4/10[c] (1.0)	5/10[c] (1.0)	9/10[b] (1.9)
Females						
Duodenum (histiocytic cellular infiltration)	0/10[a]	1/10 (1.0)	5/10[c] (1.0)	7/10[b] (1.4)	8/10[b] (1.6)	10/10[b] (1.7)
Stomach, glandular (ulcer)	0/10	0/10	0/10	0/10	0/10	10/10[b] (3.5)
Stomach, glandular (focal regenerative hyperplasia)	0/10	0/10	0/10	0/10	0/10	10/10[b] (2.0)
Stomach, glandular (focal squamous hyperplasia)	0/10	0/10	0/10	0/10	0/10	10/10[b] (2.4)
Pancreatic lymph node (ectasia)	0/10	0/10	0/10	0/10	1/10 (1.0)	10/10[b] (1.8)
Pancreatic lymph node (lymphoid hyperplasia)	0/10	0/10	2/10 (1.5)	0/10	0/10	10/10[b] (2.1)
Pancreatic lymph node (histiocytic cellular infiltration)	4/10 (1.0)	8/10 (1.4)	7/10 (1.7)	7/10 (1.3)	7/10 (1.7)	9/10[c] (1.9)
Liver (histiocytic cellular infiltration)	0/10	3/10 (1.3)	6/10[b] (1.0)	6/10[b] (1.0)	9/10[b] (1.2)	8/10[b] (1.0)
Liver (chronic focal inflammation)	3/10 (1.0)	5/10 (1.0)	2/10 (1.0)	7/10 (1.0)	2/10 (1.0)	10/10[b] (1.0)
Bone marrow (hyperplasia)	0/10	0/10	0/10	0/10	0/10	4/10[c] (1.0)

[a]Number of animals with lesion/number of animals examined; parenthesis indicate average severity grade, with 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Significantly different ($p \leq 0.01$) from the control group by Fisher's exact test.
[c]Significantly different ($p \leq 0.05$) from the control group by Fisher's exact test.

Source: NTP (2007).

In conclusion, the NTP (2007) 3-month study in F344/N rats exposed to sodium dichromate dihydrate in drinking water identified several effects of subchronic oral hexavalent chromium exposure, including changes in hematological endpoints (microcytic, hypochromic anemia), hepatotoxicity (increased serum enzyme activities, increased serum bile acids, and histopathological changes), alterations in lipid metabolism (decreased serum cholesterol and triglycerides), possible muscle damage (increased serum creatine kinase activity), and histopathological changes in GI tissues (duodenum and glandular stomach) and pancreatic lymph nodes. EPA used the results of this study to identify a LOAEL in male and female rats of 1.7 mg hexavalent chromium/kg-day; a NOAEL was not identified. In males, the LOAEL was based on observations of microcytic, hypochromic anemia (decreased Hct, Hgb, MCV, MCH) observed after 23 days and 3 months of exposure, increased serum liver enzyme activities (ALT and SDH), and histopathological changes to pancreatic lymph nodes (histiocytic cellular infiltration), all observed at daily doses ≥1.7 mg hexavalent chromium/kg-day. In females, the LOAEL was based on observations of microcytic, hypochromic anemia (decreased Hgb, MCV, MCH) observed after 23 days and 3 months of exposure, and increased serum liver enzyme activities (ALT and SDH) and bile acids, all observed at daily doses ≥1.7 mg hexavalent chromium/kg-day.

In the 3-month study in $B6C3F_1$ mice, groups of 10 males and 10 females were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 62.5, 125, 250, 500, or 1,000 mg sodium dichromate dihydrate/L (equivalent to 0, 21.8, 43.6, 87.2, 174.5, or 348 mg hexavalent chromium/L, respectively) for 3 months (NTP, 2007). Based on water consumption monitored throughout the study, NTP (2007) calculated average daily doses over the 3-month treatment duration of approximately 0, 9, 15, 26, 45, or 80 mg sodium dichromate dihydrate/kg-day (equivalent to 0, 3.1, 5.3, 9.1, 15.7, or 27.9 mg hexavalent chromium/kg-day, respectively) for both males and females. Mice were subjected to the same evaluations and procedures as those described above for "core" study rats (NTP, 2007), except that blood was not analyzed for clinical chemistry as the study in mice did not include a group of "clinical pathology" animals for evaluation after exposure durations of 5 and 23 days.

No mortalities were observed in male or female mice exposed to sodium dichromate dihydrate in drinking water for 3 months (NTP, 2007). Dose-related significant decreases were observed in final body weights in male mice, with decreases reaching 20% (compared with control values) in the 27.9 mg hexavalent chromium/kg-day group; in females, dose-related decreases in final body weight were observed at ≥5.3 mg hexavalent chromium/kg-day, with decreases reaching 13% in the 27.9 mg hexavalent chromium/kg-day group. Drinking water consumption was reduced in males at ≥5.3 mg hexavalent chromium/kg-day and in females at 27.9 mg hexavalent chromium/kg-day (statistical significance not reported). Data on food consumption were not reported. No treatment-related signs of clinical toxicity were observed throughout the study.

Results of hematological analyses showed that mice exposed to sodium dichromate dihydrate in drinking water for 3 months developed mild erythrocyte microcytosis (NTP, 2007); however, compared to hematological effects observed in rats (described above), effects in mice were less severe. In male mice, MCV and MCH were significantly decreased in all treatment groups, with maximum decreases of approximately 8%, compared to controls, in the highest dose group. In females, MCV and MCH were significantly reduced at ≥3.1 and ≥5.2 mg hexavalent chromium/kg-day, respectively, with maximum decreases of approximately 9 and 10%, respectively, compared to controls, in the highest dose group. Although statistically significant ($p < 0.05$) decreases in MCV were observed in males and females in the 3.1 mg hexavalent chromium/kg-day group, decreases were very small (1–2%, compared to controls); at doses up to 9.1 mg hexavalent chromium/kg-day, decreases in MCV were ≤5%, compared with controls. Thus, EPA did not consider the mild microcytosis observed at ≥9.1 mg hexavalent chromium/kg-day to represent a clinically significant effect. Erythrocyte counts were slightly increased (≤6%, compared with controls) at ≥5.2 mg hexavalent chromium/kg-day in females, but not in males.

Changes in organ weights in mice exposed to sodium dichromate dihydrate in drinking water for 3 months are summarized in Table 4-8 (NTP, 2007). In males, absolute liver and right kidney weights were decreased at ≥9.1 mg hexavalent chromium/kg-day, although the only significant change in relative organ weight was an increase in relative kidney weight at 27.9 mg hexavalent chromium/kg-day. In females, absolute liver weight was decreased at doses ≥15.7 mg hexavalent chromium/kg-day. Changes in weights of other organs were considered by NTP (2007) to be secondary to changes in body weight rather than due to adverse effects of treatment.

Table 4-8. Selected organ weights in male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 3 months

Organ	Treatment group (mg hexavalent chromium/kg-d)					
	0	3.1	5.3	9.1	15.7	27.9
Males						
Right kidney, absolute weight	0.28 ± 0.01[a]	0.28 ± 0.01	0.26 ± 0.01	0.26 ± 0.01[b]	0.24 ± 0.01[c]	0.26 ± 0.01[c]
Right kidney, relative weight[d]	7.25 ± 0.11	7.68 ± 0.29	7.43 ± 0.35	7.75 ± 0.20	7.76 ± 0.30	8.18 ± 0.07[c]
Liver, absolute weight	1.60 ± 0.08	1.54 ± 0.05	1.50 ± 0.05	1.40 ± 0.05[b]	1.33 ± 0.06[c]	1.34 ± 0.04[c]
Females						
Liver, absolute weight[d]	1.15 ± 0.03[a]	1.14 ± 0.04	1.06 ± 0.02	1.11 ± 0.04	1.04 ± 0.02[b]	0.99 ± 0.02[c]

[a]Values are means ± SE; n = 10 mice/group.
[b]Significantly different ($p \leq 0.05$) from the control group by Williams's or Dunnett's test.
[c]Significantly different ($p \leq 0.01$) from the control group by Williams's or Dunnett's test.
[d]Relative weight = mg organ weight/g body weight.

Source: NTP (2007).

Gross and microscopic examinations of male and female mice exposed to sodium dichromate dihydrate in drinking water for 3 months showed nonneoplastic lesions of the duodenum and mesenteric lymph nodes (NTP, 2007); incidence data are summarized in Table 4-9. In the duodenum, dose-related increases were observed in the incidence of minimal-to-mild histiocytic cellular infiltration in males and females in all treatment groups and in the incidence of minimal-to-mild epithelial hyperplasia in males and females at ≥5.3 mg hexavalent chromium/kg-day; a slight dose-related increase in severity was observed. The duodenum had short, thick duodenal villi, elongated crypts with diffuse hyperplasia, and hyperplastic epithelial cells with swollen, vacuolated cytoplasm, and increased numbers of "mitotic figures" (incidence data not reported). NTP (2007) stated that the duodenal lesions were indicative of regenerative hyperplasia subsequent to epithelial cell injury. Minimal histiocytic cellular infiltration, morphologically similar to that observed in rats (discussed above), was observed in mesenteric lymph nodes in male and female mice at ≥5.3 mg hexavalent chromium/kg-day.

Table 4-9. Incidence of nonneoplastic lesions observed in male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 3 months

Tissue (lesion type)	Treatment group (mg hexavalent chromium/kg-d)					
	0	3.1	5.3	9.1	15.7	27.9
Males						
Duodenum (histiocytic cellular infiltration)	0/10[a]	4/10[b] (1.0)	5/10[c] (1.0)	10/10[c] (1.3)	10/10[c] (1.7)	10/10[c] (1.9)
Duodenum (epithelial hyperplasia)	0/10	0/10	8/10[c] (1.3)	10/10[c] (1.8)	10/10[c] (2.1)	10/10[c] (1.8)
Mesenteric lymph node (histiocytic cellular infiltration)	0/10	0/9	4/9[b] (1.0)	6/8[c] (1.0)	3/8 (2.0)	8/10[c] (1.3)
Females						
Duodenum (histiocytic cellular infiltration)	0/10[a]	7/10[c] (1.0)	8/9[c] (1.3)	10/10[c] (1.3)	10/10[c] (1.4)	10/10[c] (1.7)
Duodenum (epithelial hyperplasia)	0/10	0/10	9/9[c] (1.1)	10/10[c] (1.1)	10/10[c] (1.5)	10/10[c] (1.4)
Mesenteric lymph node (histiocytic cellular infiltration)	0/10	0/10	6/10[c] (1.0)	6/10[c] (1.0)	4/9[b] (1.3)	9/10[c] (1.1)

[a]Number of animals with lesion/number of animals examined; parenthesis indicate average severity grade, with 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Significantly different ($p \leq 0.05$) from the control group by Fisher's exact test.
[c]Significantly different ($p \leq 0.01$) from the control group by Fisher's exact test.

Source: NTP (2007).

In conclusion, the NTP (2007) 3-month study in B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water identified adverse treatment-related hematological effects (erythrocytic microcytosis) and histopathological changes to the small intestine (duodenal epithelial hyperplasia and cellular histiocytic infiltration) and mesenteric lymph nodes (cellular histiocytic infiltration). Based on histopathological changes (i.e., histiocytic cellular infiltration) in the duodenum, EPA identified a LOAEL of 3.1 mg hexavalent chromium/kg-day for male and female mice; in both sexes, a NOAEL was not identified because the effects were observed at the lowest dose tested. Although a statistically significant decrease in MCV also was observed at 3.1 mg hexavalent chromium/kg-day in males and females, hematological effects (e.g., microcytosis) were not considered as the basis of the LOAEL, since decreases in MCV were small (1–2%) at the lowest dose tested.

Finally, NTP (2007) conducted a comparative study in three strains of mice (B6C3F$_1$, BALB/c, and *am3*-C57BL/6) on the effects of exposure to sodium dichromate dihydrate in drinking water for 3 months. This comparative study was conducted to investigate possible strain differences in mice based on results of an earlier study reporting hepatotoxicity (hepatocellular vacuolization) in BALB/c mice fed 32 mg hexavalent chromium/kg-day in the

diet as potassium dichromate (NTP 1996a); no evidence of hepatotoxicity (including histopathological changes) was observed in male or female B6C3F$_1$ mice exposed for 3 months to sodium dichromate dihydrate in drinking water at doses up to 20.9 mg hexavalent chromium/kg-day (NTP, 2007; results summarized above). In the "core" study, groups of 10 male B6C3F$_1$, 10 male BALB/c, and 5 male *am3*-C57BL/6 mice were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 62.5, 125, or 250 mg/L (equivalent to 0, 21.8, 43.6, or 87.2 mg hexavalent chromium/L, respectively) for 3 months. The *am3*-C57BL/6 strain of mice is transgenic for a gene that is sensitive to forward and reverse mutation, and an additional five males of this strain were exposed to the same concentrations of sodium dichromate dihydrate in order to conduct a mutagenesis study exploiting this capability. However, this study was not conducted due to technical problems, although blood collected from these animals was still analyzed for hematology and clinical chemistry. Based on water consumption monitored throughout the study, NTP (2007) calculated average daily doses over the 3-month treatment duration of approximately 0, 8, 15, or 25 mg sodium dichromate dihydrate/kg-day (equivalent to 0, 2.8, 5.2, or 8.7 mg hexavalent chromium/kg-day, respectively) for all strains. Animals were observed twice daily for mortality and clinical signs of toxicity; body weights were recorded weekly and water consumption was recorded at least every 4 days. Blood was collected at the end of the 3-month treatment period and analyzed for hematology and clinical chemistry, as described above for "core" study rats (NTP, 2007). At study termination, necropsies were performed on all mice, with organ weights recorded for heart, right kidney, liver (except B6C3F$_1$ mice), lung, spleen, right testis, and thymus. Microscopic examination was conducted on all gross lesions and masses and selected tissues (liver, forestomach, glandular stomach, duodenum, pancreas, kidney, and mesenteric and pancreatic lymph nodes). Sperm count and motility were assessed in all study animals, including spermatids per testis and per mg testis, spermatids per cauda and per mg cauda, sperm motility, and weights of left cauda, left epididymis, and left testis.

No mortalities were observed in male B6C3F$_1$, BALB/c, or *am3*-C57BL/6 mice exposed to sodium dichromate dihydrate in drinking water for 3 months (NTP, 2007). In the 5.2 and 8.7 mg hexavalent chromium/kg-day groups, final body weights were significantly decreased (compared to controls) by 9 and 12%, respectively, in B6C3F$_1$ mice and by 7 and 11%, respectively, in BALB/c mice. Final body weight was reduced in all treatment groups in *am3*-C57BL/6 mice, with decreases reaching 44% in the 8.7 mg hexavalent chromium/kg-day group. Water consumption was reduced at 8.7 mg hexavalent chromium/kg-day in all three strains. Data on food consumption were not reported. No treatment-related signs of clinical toxicity were observed in B6C3F$_1$ or *am3*-C57BL/6 mice. In BALB/c mice, ruffled fur was observed at 8.7 mg hexavalent chromium/kg-day.

Results of hematology analyses show that male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice exposed to sodium dichromate dihydrate in drinking water for 3 months developed mild

erythrocyte microcytosis (e.g., MCV) and small decreases in MCH, with changes observed in most treatment groups (Table 4-10) (NTP, 2007). In the 2.8 and 5.2 mg hexavalent chromium/kg-day groups, decreases in MCV and MCH were ≤7%, compared with controls, with slightly greater decreases at 8.7 mg hexavalent chromium/kg-day. Erythrocyte counts were significantly increased in $B6C3F_1$ mice (7% at 8.7 mg hexavalent chromium/kg-day) and in BALB/c mice (2 and 5% at 5.2 and 8.7 mg hexavalent chromium/kg-day, respectively), but not in *am3*-C57BL/6 mice. Hgb and Hct were decreased by approximately 5%, in *am3*-C57BL/6 mice at 8.7 mg hexavalent chromium/kg-day, compared with controls, but not in $B6C3F_1$ or BALB/c mice. Compared with hematological effects observed in rats (described previously), effects in mice were much less severe. Clinical chemistry analysis showed small increases (1.2- to 1.3-fold) in ALT at ≥5.2 mg hexavalent chromium/kg-day in BALB/c mice and a 1.9-fold increase in ALT in *am3*-C57BL/6 mice; in $B6C3F_1$ mice, no increases in serum liver enzyme activities were observed. Decreases in various absolute and relative organ weights were observed at ≥5.2 mg hexavalent chromium/kg-day. NTP (2007) considered all changes to be related to decreased body weight, except for a significant decrease (29% compared with controls; $p \leq 0.05$) in absolute thymus weight in $B6C3F_1$ mice in the 8.7 mg hexavalent chromium/kg-day group; however, relative thymus weight was not different from controls in any treatment group. No treatment-related effects were observed for reproductive tissue evaluations or other reproductive parameters, except for a significant decrease (12.4% compared to controls; $p \leq 0.01$) in absolute left testis weight in *am3*-C57BL/6 mice at 8.7 mg hexavalent chromium/kg-day; NTP (2007) stated that this change was related to decreased body weight.

Table 4-10. Hematological effects in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice exposed to sodium dichromate dihydrate in drinking water for 3 months

	Treatment group (mg hexavalent chromium/kg-d)			
	0	**2.8**	**5.2**	**8.7**
B6C3F$_1$ mice				
MCV (fL)	47.7 ± 0.2[a]	46.6 ± 0.2[b] (97.7)	46.4 ± 0.2 (97.3)	44.7 ± 0.1 (93.7)
MCH (ρg)	15.3 ± 0.1	14.9 ± 0.1[b] (93.1)	14.7 ± 0.1[b] (96.1)	14.2 ± 0.0[b] (92.8)
BALB/c mice				
MCV (fL)	44.8 ± 0.2[a]	43.8 ± 0.2[b] (97.8)	42.9 ± 0.2 (95.8)	42.6 ± 0.2 (95.1)
MCH (ρg)	15.0 ± 0.1	14.5 ± 0.1[b] (96.7)	14.2 ± 0.1[b] (94.7)	14.0 ± 0.1[b] (93.3)
***am3*-C57BL/6 mice**				
MCV (fL)	45.8 ± 0.2 [a]	44.2 ± 0.4 (96.5)	43.7 ± 0.3[b] (95.4)	40.5 ± 0.3 (88.4)
MCH (ρg)	14.4 ± 0.1	14.1 ± 0.1[b] (97.9)	13.8 ± 0.1[b] (95.8)	13.5 ± 0.2[b] (98.8)

[a]Values are means ± SE; values in parenthesis are percent of control; n = 10 mice/group, with the following exceptions: in B6C3F$_1$ mice, controls (n = 7), 2.8 mg hexavalent chromium/kg-d group (n = 9), and 5.2 mg hexavalent chromium/kg-d group (n = 9); in *am3*-C57BL/6 mice, 8.7 mg hexavalent chromium/kg-d group (n = 9).
[b]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.

Source: NTP (2007).

Microscopic examinations of gross lesions and masses and of selected tissues in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice exposed to sodium dichromate dihydrate in drinking water for 3 months showed changes to the duodenum, liver, pancreas, and mesenteric lymph nodes (NTP, 2007); incidence data are summarized in Table 4-11. Dose-related increases in the incidences of hepatic glycogen depletion and pancreatic secretory depletion were also observed; NTP (2007) stated that these lesions were likely due to depressed food consumption, which is frequently observed when water consumption is decreased. The incidence of minimal-to-mild histiocytic cellular infiltration of mesenteric lymph nodes was increased at 8.7 mg hexavalent chromium/kg-day in *am3*-C57BL/6 mice, but not in B6C3F$_1$ or BALB/c mice.

In the duodenum, dose-related increases in the incidences of minimal-to-mild histiocytic cellular infiltration and epithelial hyperplasia were observed in all strains, with histopathological changes of the duodenum observed in all exposure groups; severity increased with dose. Microscopically, lesions were similar to those described above for male and female B6C3F$_1$ mice. The incidences of histiocytic cellular infiltration and epithelial hyperplasia in the duodenum, however, were smaller in the initial 3-month study in B6C3F$_1$ mice than in this study. For example, the incidences of histiocytic cellular infiltration in the initial study with male

$B6C3F_1$ mice were 4/10 and 5/10 at the 3.1 and 5.3 mg/kg-day levels, respectively; the incidence of epithelial hyperplasia was 0/10 at 3.1 mg/kg-day (Table 4-9). However, as shown in Table 4-11, at comparable dose levels of 2.8 and 5.2 mg/kg-day, the incidences of histiocytic infiltration in male $B6C3F_1$ mice in the second study were 8/10 and 10/10, and the incidence of epithelial hyperplasia at 2.8 mg/kg-day was 4/10. The basis for these inconsistencies in the magnitude of the duodenal lesions across studies is not known.

Table 4-11. Incidence of nonneoplastic lesions observed in male $B6C3F_1$, BALB/c, and *am3*-C57BL/6 mice exposed to sodium dichromate dihydrate in drinking water for 3 months

Tissue (lesion type)	Treatment group (mg hexavalent chromium/kg-d)			
	0	2.8	5.2	8.7
$B6C3F_1$ mice				
Duodenum (histiocytic cellular infiltration)	0/10[a]	8/10[b] (1.0)	10/10[b] (1.4)	10/10[b] (2.0)
Duodenum (epithelial hyperplasia)	0/10	4/10[c] (1.0)	10/10[b] (1.1)	10/10[b] (1.6)
Liver (glycogen depletion)	1/10 (1.0)	2/10 (1.5)	9/10[b] (1.4)	10/10[b] (2.2)
Pancreas (secretory depletion)	0/10	2/10 (1.0)	7/10[b] (1.0)	9/10[b] (1.0)
BALB/c mice				
Duodenum (histiocytic cellular infiltration)	0/10[a]	4/10[c] (1.0)	8/10[b] (1.8)	10/10[b] (1.7)
Duodenum (epithelial hyperplasia)	0/10	2/10 (1.0)	10/10[b] (1.1)	10/10[b] (1.4)
Pancreas (secretory depletion)	0/10	6/10[b] (1.0)	9/10[b] (1.3)	10/10[b] (1.5)
***am3*-C57BL/6 mice**				
Duodenum (histiocytic cellular infiltration)	0/5[a]	2/5 (1.0)	5/5[b] (1.4)	4/5[c] (1.8)
Duodenum (epithelial hyperplasia)	0/5	5/5[b] (1.0)	5/5[b] (1.2)	5/5[b] (1.8)
Liver (glycogen depletion)	0/5	4/5[c] (2.0)	5/5[b] (1.6)	5/5[b] (3.8)
Pancreas (secretory depletion)	0/5	3/5 (1.0)	4/5[c] (1.0)	5/5[b] (1.6)
Mesenteric lymph node (histiocytic cellular infiltration)	0/5	0/5	0/5	4/5[c] (1.5)

[a]Number of animals with lesion/number of animals examined; parenthesis indicate average severity grade, with 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Significantly different ($p \leq 0.01$) from the control group by Fisher's exact test.
[c]Significantly different ($p \leq 0.01$) from the control group by Fisher's exact test.

Source: NTP (2007).

In conclusion, the comparative 3-month drinking water study on sodium dichromate dihydrate in male $B6C3F_1$, BALB/c, and *am3*-C57BL/6 mice showed similar effects in the three strains (NTP, 2007). A LOAEL of 2.8 mg hexavalent chromium/kg-day was identified by EPA based on histopathological changes in the duodenum in $B6C3F_1$ mice (i.e., histiocytic cellular infiltration and epithelial hyperplasia), BALB/c mice (i.e., histiocytic cellular infiltration), and *am3*-C57BL/6 mice (i.e., epithelial hyperplasia); a NOAEL was not identified because effects were observed at the lowest doses tested. Mild erythrocyte microcytosis was not considered as the basis for the LOAEL because the magnitude of decreases in MCV and MCH in the 2.8 mg hexavalent chromium/kg-day group was ≤7% compared to controls.

Quinteros et al., 2007

Quinteros et al. (2007) showed that subchronic oral exposure of rats to hexavalent chromium in drinking water decreased circulating prolactin levels. Groups of 15 male Wistar rats were exposed to drinking water containing 0 or 500 mg hexavalent chromium/L as potassium dichromate for 30 days. Based on water intake and body weights measured over the course of the study, Quinteros et al. (2007) calculated a daily dose of 73.05 mg hexavalent chromium/kg-day. At the end of the treatment period, blood was collected for analysis of prolactin and luteinizing hormone (LH), and the pituitary gland and hypothalamus were analyzed for chromium content. At the end of the 30-day treatment period, water consumption and body weight in hexavalent chromium-treated rats were decreased by 30.5 and 11.5% compared to controls. Serum prolactin levels in treated rats were decreased by approximately 59% ($p < 0.001$) compared to controls; serum levels of LH were comparable in control and treatment groups. NOAEL and LOAEL values for this study could not be identified because only one dose was evaluated and effects on other potential hexavalent chromium target tissues were not assessed.

Rafael et al., 2007

Adverse hepatic effects were reported in rats following subchronic oral exposure to hexavalent chromium, but further details of this study were not available (Rafael et al., 2007). Male Wistar rats (9 control and 19 treated) were administered drinking water containing 0 or 20 mg hexavalent chromium/L (chromium compound not reported) for 10 weeks. According to the investigators, no clinical signs of toxicity or changes in body weight were observed (data not reported). Data on drinking water consumption were not reported, and the report did not indicate if drinking water consumption was similar between control and treatment groups; thus, given this uncertainty, daily hexavalent chromium doses cannot be estimated from this study. At the end of the treatment period, serum glucose was decreased by 45% ($p = 0.0002$) and serum ALT activity was increased by 153% ($p = 0.039$), compared with controls. Serum levels of total protein, gamma glutamyl transferase, AP, cholesterol, and total bilirubin were not affected by treatment.

Microscopic examination of livers of treated mice showed increased intracellular space, "little" focal necrosis, and degenerative alteration with vascularization; fibrosis was not observed. NOAEL and LOAEL values could not be identified from this study because only one dose was evaluated and limited exposure information was provided.

Acharya et al., 2001

Acharya et al. (2001) explored whether Wistar rats demonstrated sex-specific responses to exposures to chromium and chromium plus ethanol using a study design similar to Chopra et al. (1996), but exposing male rather than female Wistar rats. Acharya et al. (2001) exposed 1.5-month-old male Wistar rats (5 or 6/group) to potassium dichromate in drinking water for 22 weeks at concentrations of 0 or 25 ppm. These dosed groups were part of a larger study to evaluate the interactive effects of ethanol and chromium. The authors reported that food and water consumption were monitored daily and each animal was weighed once a week, although these results were not reported. Using reference values for body weight and drinking water consumption (0.217 kg and 0.032 L/day, respectively) for male Wistar rats (U.S. EPA, 1988), doses of 0 and 1.5 mg hexavalent chromium/kg-day were estimated by EPA. At study termination, animals were sacrificed and blood samples were collected for analysis of serum enzyme activities. Liver and kidney tissues were examined for histopathological changes, and liver homogenates were used to measure total triglycerides, total cholesterol, glycogen, and total GSH.

Serum aspartate aminotransferase (AST) and ALT levels were statistically significantly elevated (approximately twofold) in chromium-treated rats compared to controls. Serum succinate dehydrogenase, AP, and acid phosphatase (AcP) in chromium-treated rats were not significantly different from the controls. Liver total triglyceride and liver glycogen levels were significantly reduced in chromium-treated rats (by approximately 40 and 20%, respectively). There was a significant increase in liver total cholesterol levels (approximately 10%) in chromium-treated rats. Liver GSH levels in chromium-treated rats were similar to controls.

Histopathological examination of the livers of chromium-treated animals showed altered hepatic architecture in the periportal area, with increased sinusoidal space, vacuolation, and necrosis. Histopathological examination of the kidneys in chromium-treated rats revealed vacuolation in glomeruli, degeneration of the basement membrane, and renal tubular epithelial degeneration. No information regarding the number of animals examined or the number of animals displaying histopathology was provided. The only dose tested in this study, 1.5 mg hexavalent chromium/kg-day, was identified by EPA as a LOAEL. A NOAEL was not identified because effects were seen at the only dose tested.

Asmatullah and Noreen, 1999

Asmatullah and Noreen (1999) studied the effects of subchronic exposure to hexavalent chromium on growth rate and hepatic histological structure in mice. Groups of male albino Swiss mice (nine per group) were exposed to drinking water containing 0, 500, 750, 1,000, 1,500, or 2,000 mg potassium dichromate/L (equivalent to 0, 177, 265, 353, 530, or 706 mg hexavalent chromium/L, respectively) for 8 weeks. Data on drinking water consumption were not reported; based on findings of other studies (NTP, 2008, 2007) showing decreased drinking water consumption and body weight in animals treated with drinking water containing ≥30 mg hexavalent chromium/L, daily doses of hexavalent chromium cannot be accurately estimated for this study. Body weights and feed consumption were recorded weekly. At the end of the treatment period, organ weights were determined for liver, heart, and kidney, and microscopic examination of the liver was conducted. During the last 2 weeks of treatment, body weights were decreased in all treatment groups, with decreases ranging from 9 to 29%, compared with controls; decreases in body weight were accompanied by similar decreases in feed intake in all treatment groups. After 8 weeks of treatment, absolute wet and dry weights of liver and heart were increased in all treatment groups, although the magnitude of these increases did not exhibit dose-dependence. No consistent pattern of change was observed for wet or dry weight of the heart. Relative organ weights were not reported. Histopathological changes in the liver were observed, with severity increasing with dose (but incidence data were not reported). At 265 mg hexavalent chromium/L, an increase in the sinusoidal space was observed; at 353 and 530 mg hexavalent chromium/L, hepatic cirrhosis and increased sinusoidal space were observed, with severity increasing with dose; and at 706 mg hexavalent chromium/L, increased sinusoidal space, cirrhosis and nuclear pyknosis (a marker for apoptosis) were observed. Results of microscopic examination of the liver in mice treated with 177 mg hexavalent chromium/L were not reported. A NOAEL or LOAEL could not be identified by EPA from this study.

Chopra et al., 1996

Chopra et al. (1996) exposed 50-day-old female Wistar rats (5 or 6/group) to potassium dichromate in drinking water for 22 weeks at concentrations of 0 or 25 ppm. These dose groups were part of a larger study designed to evaluate the interactive effects of ethanol and chromium. The authors reported that food and water consumption were monitored daily and each animal was weighed once a week, although these results were not reported. Using reference values for body weight and drinking water consumption (0.156 kg and 0.025 L/day, respectively) for female Wistar rats (U.S. EPA, 1988), doses of 0 and 1.4 mg hexavalent chromium/kg-day were estimated by EPA. At study termination, animals were sacrificed and blood samples were collected for analysis of serum enzyme activities and serum triglycerides, cholesterol, and glucose. A kidney homogenate was used to measure GSH, and a liver homogenate was used to

measure triglycerides, cholesterol, glycogen, GSH, and lipid peroxidation. Liver and kidney tissues were examined for histopathological changes.

Terminal body weights in chromium-treated rats were not significantly different from the controls. The liver to body weight ratio in chromium-treated rats was statistically significantly increased (approximately twofold) over the controls. Serum SDH levels were significantly lower (by approximately 20%) in chromium-treated rats compared to the controls, whereas AST, ALT, AP, and AcP were statistically significantly increased (approximately two- to threefold). Serum triglycerides and glucose were statistically significantly increased (approximately threefold) in chromium-treated rats; serum cholesterol was significantly reduced (approximately twofold). Analysis of liver homogenates revealed that chromium treatment resulted in reduced liver glycogen (by approximately twofold); levels of liver cholesterol, GSH, and lipid peroxidation (as measured by diene conjugation) did not differ from the controls. Kidney GSH in chromium-treated rats was statistically significantly lower than the controls (approximately 2.5-fold).

Histopathological examination of the liver of chromium-treated animals showed altered hepatic architecture in the periportal area, with increased sinusoidal space, vacuolation, and necrosis. Histopathological examination of the kidneys in chromium-treated rats revealed significant damage to renal tubules and the Bowman's capsule and degeneration of the basement membrane. No information regarding the number of animals examined or the number of animals displaying histopathology was provided. The only dose tested in this study, 1.4 mg hexavalent chromium/kg-day, was identified as a LOAEL by EPA. A NOAEL was not identified because effects were observed at the only dose tested.

Vyskocil et al., 1993

Alterations in renal function, as assessed by urinalysis, were observed in rats exposed to oral potassium chromate for up to 6 months (Vyskocil et al., 1993). Groups of Wistar rats (20/sex/group) were exposed to drinking water containing 0 or 25 mg hexavalent chromium/L. Based on water consumption, which was comparable between control and treatment groups, Vyskocil et al. (1993) calculated average daily hexavalent chromium doses of 2.18 and 2.47 mg hexavalent chromium/kg-day in males and females, respectively, during the first 3 months of exposure, and 1.40 and 1.76 mg hexavalent chromium/kg-day in males and females, respectively, during the second 3 months of exposure. After 3 or 6 months of exposure, urine was collected from 10 rats/sex/group and analyzed for total protein, albumin, β_2-microglobulin, β-N-acetyl-D-glucosamine, and lactate dehydrogenase and lysozyme activities, and body and kidney weights were determined. Water consumption was monitored throughout the study. No effects on body weight gain or kidney weight were observed. In male rats, results of urinalysis did not show any treatment-related effects. In females, urinary albumin excretion, a marker of glomerular function, was significantly increased by approximately twofold ($p < 0.05$), compared to controls, at both 3 months and 6 months. Urinary β_2-microglobulin, a marker of renal tubular

dysfunction, was increased by twofold ($p < 0.05$) at 3 months and by 1.4-fold at 6 months (not statistically significant) compared to controls. Gross or microscopic examinations of kidneys were not conducted. NOAEL and LOAEL values from this study could not be identified because only one dose was evaluated and effects on other potential hexavalent chromium target tissues were not assessed.

4.2.2. Chronic Oral Exposure

NTP, 2008

NTP (2008) conducted a 2-year toxicology and carcinogenicity study of sodium dichromate dihydrate in drinking water in rats and mice. Groups of F344/N rats ("core" study animals; 50/sex/group) were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 14.3, 57.3, 172, or 516 mg sodium dichromate dihydrate/L (equivalent to 0, 5, 20, 60, or 180 mg hexavalent chromium/L, respectively). Based on water consumption measured throughout the study, NTP (2008) calculated average daily doses over the 2-year treatment duration of approximately 0, 0.6, 2.2, 6, or 17 mg sodium dichromate dihydrate/kg-day for males (equivalent to 0, 0.21, 0.77, 2.1, or 5.9 mg hexavalent chromium/kg-day, respectively) and 0.7, 2.7, 7, and 20 mg sodium dichromate dihydrate/kg-day for females (equivalent to 0, 0.24, 0.94, 2.4, or 7.0 mg hexavalent chromium/kg-day, respectively). Animals were observed twice daily for mortality and clinical signs of toxicity; after 5 weeks of treatment, clinical signs were recorded at 4-week intervals. Body weights were recorded weekly for the first 13 weeks, and then at 4-week intervals for the duration of the study. Water consumption was recorded weekly for the first 13 weeks of treatment and then every 4 weeks. At the end of the 2-year treatment period, complete necropsies and microscopic examinations of comprehensive tissues were performed on all "core" study animals. An additional "special study" group of male rats (10/group) was exposed to the same drinking water concentrations as "core" animals for up to 53 weeks. For the "special study" rats only, blood was collected on days 4 and 22 and at 3, 6, and 12 months for hematology (i.e., Hct; Hgb concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; MCV; MCH; mean cell hemoglobin concentration [MCHC]; and leukocyte count and differentials) and clinical chemistry (i.e., urea nitrogen, creatinine, total protein, albumin, ALT, AP, creatine kinase, sorbitol dehydrogenase, bile acids) analyses. At the end of the 53-week treatment period, "special study" animals were evaluated for chromium tissue distribution (see Section 3.2 for the results of this study).

Survival rates of exposed "core" study rats were similar to controls (NTP, 2008). Throughout the study, water consumption was decreased in the two highest dose groups compared to controls. During the second year of the study, water consumption in the two highest dose groups in males was decreased by 15 and 22%, respectively, and by 15 and 27%, respectively, in females (statistical significance not reported). No data on food consumption were reported. At the end of the 2-year treatment period, body weight was decreased in males

and females in the highest dose group by 12 and 11%, respectively, compared with controls (statistical significance not reported). NTP (2008) suggested that decreased body weights in the highest dose group may have been partially due to decreased water consumption (due to decreased palatability), rather than an adverse effect of sodium dichromate dihydrate. No treatment-related signs of clinical toxicity were observed throughout the study.

Results of hematologic analyses in "special study" male rats showed that exposure to sodium dichromate dihydrate in drinking water produced microcytic, hypochromic anemia, characterized by decreases in MCV, Hct, Hgb, MCH, and MCHC (NTP, 2008). The severity of microcytic, hypochromic anemia exhibited duration- and dose-dependence, with peak effects occurring at 22 days (Table 4-12). After 4 days of exposure, small changes were observed in several hematological parameters; however, decreases in all treatment groups were ≤5%, compared to controls. More severe effects were observed after 22 days of treatment, with significant decreases in MCV, Hct, and Hgb at ≥0.77 mg hexavalent chromium/kg-day. At 5.9 mg hexavalent chromium/kg-day, MCV, Hct, and Hgb decreased to approximately 76, 73, and 65% of control values, respectively; reticulocyte and nucleated erythrocyte counts were increased by approximately 66% ($p \leq 0.01$) and 600% ($p \leq 0.01$), respectively, compared to controls, indicating compensatory hematopoiesis. Blood smears showed evidence of erythrocyte injury or increased turnover, including poikilocytes, erythrocyte fragments, and keratocytes (incidence data not reported). Similar effects were observed after 3 months of treatment, although severity at 3 months was generally less than that observed at 22 days. Severity was further decreased after 6 and 12 months of exposure; at 12 months, affected parameters were generally only decreased by ≤5%, compared to controls. Results of hematological analyses show that exposure of rats to sodium dichromate dihydrate in drinking water produced microcytic, hypochromic anemia at subchronic exposure durations (22 days to 3 months), but that severity decreased with increasing exposure duration (6–12 months).

Table 4-12. Hematological effects in male F344/N rats exposed to sodium dichromate dihydrate in drinking water for up to 12 months

Hematological parameter	Time on treatment	Treatment group (mg hexavalent chromium/kg-d)				
		0	0.21	0.77	2.1	5.9
MCV (fL)	D 22	59.5 ± 0.4[a]	58.6 ± 0.5 (98.5)	54.9 ± 0.5[b] (92.3)	47.4 ± 0.4[b] (80.0)	45.0 ± 0.7[b] (75.6)
	Mo 3	48.6 ± 0.2	48.3 ± 0.2 (99.4)	47.3 ± 0.2[b] (97.3)	45.7 ± 0.2[b] (94.0)	39.2 ± 0.6[b] (80.7)
	Mo 6	49.8 ± 0.1	49.5 ± 0.1 (99.4)	48.6 ± 0.1[b] (97.6)	47.8 ± 0.2[b] (96.0)	45.4 ± 0.5[b] (91.2)
	Mo 12	52.6 ± 0.2	52.4 ± 0.2 (99.6)	51.9 ± 0.3 (98.7)	51.4 ± 0.3[b] (97.7)	49.9 ± 0.2[b] (94.9)
Hct (%)	D 22	46.0 ± 1.1	44.4 ± 0.4 (96.5)	43.2 ± 0.6[c] (93.9)	38.7 ± 0.6[b] (84.1)	33.5 ± 0.8[b] (72.8)

Table 4-12. Hematological effects in male F344/N rats exposed to sodium dichromate dihydrate in drinking water for up to 12 months

Hematological parameter	Time on treatment	Treatment group (mg hexavalent chromium/kg-d)				
		0	0.21	0.77	2.1	5.9
	Mo 3	45.3 ± 0.4	44.5 ± 0.3 (98.2)	44.5 ± 0.4 (98.2)	44.1 ± 0.5 (97.4)	41.0 ± 0.5[b] (90.5)
	Mo 6	45.9 ± 0.4	45.7 ± 0.5 (99.6)	45.5 ± 0.4 (99.1)	45.5 ± 0.5 (99.1)	45.0 ± 0.3 (98.0)
	Mo 12	47.6 ± 0.5	46.6 ± 0.4 (97.9)	47.4 ± 0.5 (99.6)	47.7 ± 0.4 (100.2)	47.3 ± 0.4 (99.4)
Hgb (g/dL)	D 22	15.5 ± 0.3	15.1 ± 0.2 (97.4)	14.2 ± 0.2[b] (91.6)	12.0 ± 0.3[b] (77.4)	10.1 ± 0.2[b] (65.2)
	Mo 3	15.1 ± 0.1	14.9 ± 0.1 (98.7)	14.9 ± 0.2 (98.7)	14.6 ± 0.2[c] (96.7)	12.9 ± 0.2[b] (85.4)
	Mo 6	15.2 ± 0.1	15.2 ± 0.2 (100)	15.0 ± 0.2 (98.7)	14.9 ± 0.1 (98.0)	14.5 ± 0.1[b] (95.4)
	Mo 12	15.8 ± 0.2	15.4 ± 0.2 (97.5)	15.6 ± 0.2 (98.7)	15.6 ± 0.2 (98.7)	15.3 ± 0.1[c] (96.8)
MCH (ρg)	D 22	19.8 ± 0.1	19.5 ± 0.2 (98.5)	17.7 ± 0.2[b] (89.4)	14.8 ± 0.2[b] (74.7)	16.3 ± 0.5[b] (82.3)
	Mo 3	16.2 ± 0.1	16.2 ± 0.1 (100)	15.7 ± 0.0[b] (96.9)	15.0 ± 0.1[b] (92.6)	11.9 ± 0.3[b] (73.5)
	Mo 6	16.3 ± 0.1	16.1 ± 0.1 (98.8)	15.7 ± 0.1[b] (96.3)	15.3 ± 0.1[b] (93.9)	14.3 ± 0.2[b] (87.7)
	Mo 12	17.0 ± 0.1	16.8 ± 0.1 (98.8)	16.6 ± 0.1[c] (97.6)	16.2 ± 0.1[b] (95.3)	15.7 ± 0.1[b] (92.4)
MCHC (g/dL)	D 22	33.3 ± 0.1	33.3 ± 0.1 (100)	32.2 ± 0.2 (96.7)	31.2 ± 0.2[b] (93.7)	36.2 ± 0.8 (108.7)
	Mo 3	33.4 ± 0.1	33.5 ± 0.2 (100.3)	33.2 ± 0.1 (99.4)	32.7 ± 0.1[b] (97.9)	30.2 ± 0.3[b] (90.4)
	Mo 6	32.7 ± 0.1	32.5 ± 0.1 (99.4)	32.3 ± 0.1[c] (98.8)	32.1 ± 0.1[b] (98.2)	31.6 ± 0.2[b] (96.6)
	Mo 12	32.3 ± 0.2	32.1 ± 0.3 (99.4)	32.0 ± 0.2 (99.1)	31.6 ± 0.2[c] (97.8)	31.5 ± 0.2[c] (97.5)
Erythrocyte count (10^6/μL)	D 22	7.80 ± 0.13	7.74 ± 0.15 (99.2)	8.06 ± 0.16 (103.3)	8.10 ± 0.14 (103.8)	6.21 ± 0.13[b] (79.6)
	Mo 3	9.28 ± 0.05	9.24 ± 0.06 (99.6)	9.46 ± 0.11 (101.9)	9.75 ± 0.11[b] (105.1)	10.93 ± 0.16[b] (117.7)
	Mo 6	9.34 ± 0.06	9.43 ± 0.08 (101.0)	9.54 ± 0.11 (102.1)	9.71 ± 0.08[b] (104.0)	10.15 ± 0.13[b] (108.7)
	Mo 12	9.27 ± 0.10	9.17 ± 0.07 (98.9)	9.40 ± 0.12 (101.4)	9.61 ± 0.11 (103.7)	9.74 ± 0.08[b] (105.1)

[a]Values are means ± SE; values in parenthesis are percent of control; n = 10 rats/group, with the following exceptions: control group on d 4 (n = 9), 0.77 mg hexavalent chromium/kg-d group on d 4 (n = 9), and 2.1 mg hexavalent chromium/kg-d group in mo 12 (n = 8).
[b]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.
[c]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.

Source: NTP (2007).

Results of clinical chemistry analyses in "special study" male rats (clinical chemistry was not assessed in female rats) showed that exposure to sodium dichromate dihydrate in drinking water produced dose-dependent increases in serum ALT activity (NTP, 2008). Significant increases in serum ALT activity were observed at 4 days and 6 months in rats treated with ≥2.1 mg hexavalent chromium/kg-day and at 22 days and 3 and 12 months at ≥0.77 mg hexavalent chromium/kg-day (Table 4-13). Serum ALT enzyme activity reached maximum increases (approximately 170–260% of control values) in rats treated for 3–12 months at daily doses of ≥2.1 mg hexavalent chromium/kg-day. In rats treated for 12 months with 2.1 and 5.9 mg hexavalent chromium/kg-day, serum SDH activity was 164 and 173% of control values, respectively; however, no increases in SDH activity were observed at other doses or time points. No increases in serum AP activity were observed in any treatment group throughout the 12-month treatment period. Increased serum ALT activity is consistent with histopathological findings of minimal chronic inflammation of the liver observed in "core" study animals (discussed below); however, because other clinical chemistry markers of hepatic damage were not observed, NTP (2008) suggested that increased serum ALT activity may reflect enzyme induction rather than hepatocellular damage. Changes in other clinical chemistry outcomes were generally <5% compared to controls and did not exhibit dose- or duration-dependence.

Table 4-13. Serum ALT activity in male F344/N rats exposed to sodium dichromate dihydrate in drinking water for up to 12 months

	Treatment group (mg hexavalent chromium/kg-d)				
Time on treatment	**0**	**0.21**	**0.77**	**2.1**	**5.9**
D 4	54 ± 2[a]	53 ± 2 (98)	60 ± 3 (113)	68 ± 1[b] (126)	70 ± 2[b] (130)
D 22	45 ± 1	46 ± 1 (102)	58 ± 2[b] (129)	75 ± 3[b] (167)	73 ± 4[b] (162)
Mo 3	82 ± 4	82 ± 12 (100)	135 ± 18[c] (165)	176 ± 13[b] (215)	216 ± 21[b] (263)
Mo 6	122 ± 15	114 ± 9 (93)	150 ± 12 (123)	238 ± 2[b] (195)	210 ± 12[b] (172)
Mo 12	102 ± 6	107 ± 8 (105)	135 ± 10[c] (132)	261 ± 23[b] (256)	223 ± 15[b] (219)

[a]Values are means ± SE; values in parenthesis are percent of control; n = 10 rats/group, with the following exception of 0.77 mg hexavalent chromium/kg-d group on d 4 (n = 9). Note: clinical chemistry was not assessed in female rats.
[b]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.
[c]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.

Source: NTP (2008).

Gross and microscopic examinations of "core" study rats exposed to sodium dichromate dihydrate in drinking water for 2 years showed nonneoplastic lesions of the small intestine (duodenum), liver, and lymph nodes in both sexes, nonneoplastic lesions of the salivary gland in females, and neoplastic lesions of the oral cavity in both sexes (NTP, 2008). Incidence data for nonneoplastic lesions are summarized in Table 4-14. The incidence of minimal-to-mild cellular histiocytic infiltration of the duodenum was significantly increased in males and females at ≥0.77 and ≥2.4 mg hexavalent chromium/kg-day, respectively, compared with controls; increases in both sexes were dose-related. Duodenal histiocytic infiltrate was characterized by single or clusters of macrophages in the lamina propria of the duodenal villi. Based on incidence data, males appeared more sensitive than females to hexavalent chromium-induced nonneoplastic changes to the small intestine.

Table 4-14. Incidence of nonneoplastic lesions observed in male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years

Tissue (lesion type)	Treatment group (mg hexavalent chromium/kg-d)				
	0	**0.21**	**0.77**	**2.1**	**5.9**
Males					
Liver (histiocytic cellular infiltration)	1/50[a] (1.0)	0/50	2/49 (1.0)	5/50 (1.4)	34/49[b] (1.4)
Liver (chronic inflammation)	19/50 (1.1)	25/50 (1.2)	21/49 (1.3)	28/50[c] (1.1)	26/49 (1.3)
Liver (basophilic focus)	22/50	28/50	29/49[c]	32/50[c]	30/49
Small intestine, duodenum (histiocytic cellular infiltration)	0/48	0/48	6/47[c] (1.2)	36/46[b] (1.1)	47/48[b] (1.5)
Lymph node, mesenteric (histiocytic cellular infiltration)	13/49 (2.0)	11/50 (1.5)	30/49 (1.9)	39/50[b] (2.1)	41/49 [b] (2.1)
Lymph node, mesenteric (hemorrhage)	2/49 (1.5)	7/50 (1.1)	9/49[c] (1.3)	8/50[c] (1.1)	17/49[b] (1.3)
Tissue (lesion type)	**Treatment group (mg hexavalent chromium/kg-d)**				
	0	**0.24**	**0.94**	**2.4**	**7.0**
Females					
Liver (histiocytic cellular infiltration)	1/50[a] (1.0)	5/50 (1.0)	21/50[b] (1.3)	42/50[b] (2.0)	47/50[b] (2.6)
Liver (chronic inflammation)	12/50 (1.3)	21/50[c] (1.2)	28/50[b] (1.3)	35/50[b] (1.6)	39/50[b] (2.1)
Liver (fatty change)	3/50 (3.3)	7/50 (3.6)	10/50[c] (2.5)	13/50[b] (2.5)	16/50[b] (2.8)
Liver (clear cell focus)	7/50	5/50	7/50	20/50[b]	7/50
Small intestine, duodenum (histiocytic cellular infiltration)	0/46	0/49	1/48 (1.0)	30/46[b] (1.0)	47/50[b] (1.2)
Lymph node, mesenteric (histiocytic cellular infiltration)	21/50 (1.7)	18/50 (1.4)	27/50 (1.5)	36/50[b] (2.0)	42/50[b] (2.4)
Lymph node, mesenteric (hemorrhage)	11/50 (1.1)	13/50 (1.3)	16/50 (1.3)	14/50 (1.1)	21/50[c] (1.3)
Lymph node, pancreatic (histiocytic cellular infiltration)	17/29 (2.0)	20/36 (1.9)	23/30 (2.6)	32/34[b] (2.8)	27/33 (3.0)
Salivary gland (atrophy)	9/50 (1.3)	7/50 (1.4)	10/50 (1.2)	17/50[c] (1.4)	17/50 (2.1)

[a]Number of animals with lesion/number of animals examined; parenthesis indicate average severity grade, with 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Significantly different ($p \leq 0.01$) from the control group by the Poly-3 test.
[c]Significantly different ($p \leq 0.05$) from the control group by the Poly-3 test.

Source: NTP (2008).

Significant findings in the liver included histiocytic cellular infiltration, chronic inflammation, fatty change, basophilic foci, and clear cell foci. The incidence of histiocytic cellular inflammation, which was mild-to-moderate in severity and characterized by clusters of macrophages in parenchymal and portal areas, was significantly increased in males and females at 5.9 and ≥0.94 mg hexavalent chromium/kg-day, respectively (Table 4-14); in females, increases in incidence and severity were dose-dependent. Increased minimal-to-mild hepatic inflammation was observed in males at 2.1 mg hexavalent chromium/kg-day and in females in all treatment groups, with dose-dependent increases in incidence and severity in females. NTP (2008) noted that chronic inflammation is a typical hepatic lesion observed in aged rats; however, exposure to sodium dichromate dihydrate appeared to enhance development of this lesion. An increase in the incidence of mild-to-moderate fatty change was observed only in females at ≥0.94 mg hexavalent chromium/kg-day. Morphologically, fatty change was characterized by hepatocytes with fat-containing cytoplasmic vacuoles. The incidence of basophilic foci was increased in males only at 0.77 and 2.1 mg hexavalent chromium/kg-day, and the incidence of clear cell foci was increased in females at 2.4 mg hexavalent chromium/kg-day. Based on the dose-response data for histopathological changes of the liver, female rats appear more sensitive to hexavalent chromium than male rats to hepatic effects of sodium dichromate dihydrate.

In lymph nodes, lesions were observed in mesenteric lymph nodes (histiocytic cellular infiltration and hemorrhage) in both sexes and in pancreatic lymph nodes (histiocytic cellular infiltration) in females only. The incidence of histiocytic cellular infiltration in mesenteric lymph nodes was significantly elevated in both sexes at the two highest doses (2.1 and 5.9 mg hexavalent chromium/kg-day in males and 2.4 and 7.0 mg hexavalent chromium/kg-day in females). The incidence of mesenteric lymph node hemorrhage was significantly increased in males at ≥0.77 mg hexavalent chromium/kg-day (i.e., the three highest doses) and in females at 7.0 mg hexavalent chromium/kg-day (i.e., the highest dose). In males, the severity of histiocytic cellular infiltration and hemorrhage of mesenteric lymph nodes was minimal-to-mild in all groups, but severity of histiocytic cellular infiltration was slightly increased at ≥2.4 mg hexavalent chromium/kg-day. The incidence of cellular histiocytic infiltration of pancreatic lymph nodes was significantly increased in females in the 2.4 mg hexavalent chromium/kg-day group only, with severity increased at ≥0.94 mg hexavalent chromium/kg-day group. Morphologically, histiocytic cellular infiltrate of the lymph nodes was similar to that observed in the liver, with random clusters of macrophages located in the cortex and medullary sinuses; in mesenteric lymph nodes, some clusters merged to form sheets that replaced the parenchyma. NTP (2008) suggested that mesenteric lymph node hemorrhage may have resulted from histiocytic infiltration. A significant increase in the incidence of minimal-to-mild salivary gland atrophy, appearing as single focal lesions, was observed in females in the 2.4 mg hexavalent chromium/kg-day group only, compared with controls. NTP (2008) noted that atrophy is an age-

related change commonly observed in rats and that the biological significance of salivary atrophy in female rats chronically treated with 2.4 mg hexavalent chromium/kg-day group is unknown.

Incidence data for neoplastic lesions of the oral cavity in male and female rats exposed to sodium dichromate dihydrate in drinking water for 2 years are summarized in Table 4-15 (NTP, 2008). Neoplasms observed in the oral cavity of treated rats were squamous cell carcinoma of the oral mucosa (both sexes), squamous cell papilloma of the oral mucosa (males only), squamous cell carcinoma of the tongue (both sexes), and squamous cell papilloma and carcinoma of the tongue (both sexes). The incidences of squamous cell carcinoma of the oral mucosa (13.6%) and of combined squamous cell papilloma or carcinoma (15.7%) of the oral mucosa were significantly increased in male rats treated with 5.9 mg hexavalent chromium/kg-day, compared with controls. The incidences of squamous cell carcinoma of the oral mucosa (23.9%) and of combined squamous cell carcinoma of the oral mucosa or tongue (23.9%) were significantly increased in females treated with 7.0 mg hexavalent chromium/kg-day, compared with controls. The incidences of other neoplastic lesions of the oral cavity were not significantly increased in any treatment group in males or females compared with controls, although the incidence of squamous cell carcinoma of the oral mucosa in female rats in the 2.4 mg hexavalent chromium/kg-day group (4.6%) exceeded that of historical controls (0/300 in drinking water studies; 5/1,400 by all routes). Other neoplasms observed in treated rats included pancreatic acinar adenoma and benign pheochromocytomas in males and mononuclear cell leukemia in females (Table 4-16). However, the incidence of these neoplasms did not exhibit dose-dependence. Thus, NTP (2008) concluded that the relationship of neoplastic changes in other tissues (e.g., not of the oral cavity) to exposure to sodium dichromate dihydrate was uncertain.

Table 4-15. Incidence of neoplastic lesions observed in the oral cavity of male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years

	Treatment group (mg hexavalent chromium/kg-d)				
Neoplasm type	**0**	**0.21**	**0.77**	**2.1**	**5.9**
Males					
Oral mucosa, squamous cell papilloma					
Overall rate[a,b]	0/50 (0%)	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/49 (2%)
Oral mucosa, squamous cell carcinoma					
Overall rate[a]	0/50 (0%)	0/50 (0%)	0/49 (0%)	0/50 (0%)	6/49 (12%) [543]
Adjusted rate[c]	0% $p < 0.001$	0%	0%	0%	13.6% $p = 0.015$
Tongue, squamous cell papilloma					
Overall rate[a,b]	0/50 (0%)	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/49 (2%)
Tongue, squamous cell carcinoma					
Overall rate[a]	0/50 (0%)	1/50 (2%)	0/49 (0%)	0/50 (0%)	0/49 (0%)
Oral mucosa or tongue, squamous cell papilloma or carcinoma					
Overall rate[a]	0/50 (0%)	1/50 (2%) [729T]	0/49 (0%)	0/50 (0%)	7/49 (14.5%) [543]
Adjusted rate[c]	0% $p < 0.001$	2.4%	0%	0%	15.7% $p = 0.007$
	Treatment group (mg hexavalent chromium/kg-d)				
Neoplasm type	**0**	**0.24**	**0.94**	**2.4**	**7.0**
Females					
Oral mucosa, squamous cell carcinoma					
Overall rate[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%) [646]	11/50 (22%) [506]
Adjusted rate[c]	0% $p < 0.001$	0%	0%	4.6%	23.9% $p < 0.001$
Tongue, squamous cell papilloma					
Overall rate[a,b]	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Tongue, squamous cell carcinoma					
Overall rate[a,b]	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)

Table 4-15. Incidence of neoplastic lesions observed in the oral cavity of male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years

Neoplasm type	Treatment group (mg hexavalent chromium/kg-d)				
	0	0.24	0.94	2.4	7.0
Females					
Oral mucosa or tongue, squamous cell papilloma or carcinoma					
Overall rate[a]	1/50 (2%) [618]	1/50 (2%) [729T]	0/50 (0%)	2/50 (4%) [646]	11/50 (22%) [506]
Adjusted rate[c]	2.2% $p < 0.001$	2.3%	0%	4.6%	23.9% $p = 0.002$

[a]Overall rate: number of animals with lesion/number of animals examined; parenthesis are the percent of animals examined with lesion; brackets are days to first incidence; T: observed at terminal sacrifice. *p*-Value under treatment group incidence data indicates statistically significant Poly-3 test for pairwise comparison between control and exposed group. Statistical analysis using overall rates was only conducted if adjusted rates were not determined.
[b]Adjusted rate not reported.
[c]Adjusted rate: Poly-3 estimated neoplasm incidence (expressed as percent of animals with neoplasm) adjusted for intercurrent mortality. *p*-Value under control group indicates statistically significant positive Poly-3 trend test. *p*-Value under treatment group incidence data indicates statistically significant Poly-3 test for pairwise comparison between control and exposed groups, using adjusted rates.

Source: NTP (2008).

Table 4-16. Neoplastic lesions in other tissues (e.g., nonoral cavity) in F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years

Neoplasm type	Treatment group (mg hexavalent chromium/kg-d)				
	0	0.21	0.77	2.1	5.9
Males					
Pancreatic acinar adenoma	1/50[a,b]	2/50	6/49[c]	2/50	2/49
Benign pheochromocytoma (adrenal medulla)	6/49[a,b]	13/50[c]	14/49[c]	5/50	4/49
Neoplasm type	**Treatment group (mg hexavalent chromium/kg-d)**				
	0	**0.24**	**0.94**	**2.4**	**7.0**
Females					
Mononuclear cell leukemia	8/50[a,b]	18/50[c]	13/50	7/50	11/50

[a]Number of animals with lesion/number of animals examined.
[b]Not statistically significant for positive trend ($p > 0.05$) by the Poly-3 test.
[c]Significantly different from controls by the Poly-3 test ($p < 0.05$).

Source: NTP (2008).

In conclusion, from the NTP (2008) 2-year drinking water toxicology and carcinogenicity study on sodium dichromate dihydrate, EPA identified NOAEL and LOAEL values for noncancer effects in male rats of 0.21 and 0.77 mg hexavalent chromium/kg-day, respectively,

based on increased incidences of nonneoplastic histopathological changes to the liver (basophilic foci), duodenum (histiocytic cellular infiltrate), and mesenteric lymph nodes (histiocytic cellular infiltrate and hemorrhage). Although hematological effects indicative of microcytic, hypochromic anemia were observed in male rats exposed to ≥ 0.77 mg hexavalent chromium/kg-day from 4 days to 6 months, the severity of effects decreased over time, such that only small changes (<5%) were observed at ≥ 2.1 mg hexavalent chromium/kg-day after 12 months of exposure; therefore, hematological effects were not considered by EPA as the basis for the chronic NOAEL in male rats. In female rats, a LOAEL for noncancer effects of 0.24 mg hexavalent chromium/kg-day was identified by EPA based on the increased incidence of chronic inflammation of the liver (observed in all treatment groups); a NOAEL was not identified because these liver effects were seen at the lowest dose tested. In addition to noncancer effects, exposure of rats to sodium dichromate dihydrate in drinking water for 2 years resulted in a significant increase in squamous epithelial neoplasms of the oral mucosa and tongue at the highest exposure level (average daily doses of 5.9 and 7.0 mg hexavalent chromium/kg-day in males and females, respectively), but not at the three lower exposure levels. NTP (2008) concluded that results from this study provide clear evidence of carcinogenic activity of sodium dichromate dihydrate in male and female F344/N rats based on increased incidences of squamous cell neoplasms of the oral cavity.

$B6C3F_1$ mice were exposed to sodium dichromate dihydrate in drinking water for up to 2 years (NTP, 2008). Groups of 50 male mice (male "core" study animals) were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 14.3, 28.6, 85.7, or 257.4 mg sodium dichromate dihydrate/L (equivalent to 0, 5, 10, 30, or 90 mg hexavalent chromium/L, respectively). Based on water consumption measured throughout the study, NTP (2008) calculated average daily doses for males over the 2-year treatment duration of approximately 0, 1.1, 2.6, 7, or 17 mg sodium dichromate dihydrate/kg-day (equivalent to 0, 0.38, 0.91, 2.4, or 5.9 mg hexavalent chromium/kg-day, respectively). Groups of 50 female mice (female "core" study animals) were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 14.3, 57.3, 172, or 516 mg sodium dichromate dihydrate/L (equivalent to 0, 5, 20, 50, or 190 mg hexavalent chromium/L, respectively). Based on water consumption measured throughout the study, NTP (2008) calculated average daily doses for females over the 2-year treatment duration of approximately 0, 1.1, 3.9, 9, or 25 mg sodium dichromate dihydrate/kg-day (equivalent to 0, 0.38, 1.4, 3.1, or 8.7 mg hexavalent chromium/kg-day, respectively). "Core" study mice were subjected to the same evaluations and procedures as those described above for "core" study rats (NTP, 2008). An additional "special study" group of female mice (10/group) were exposed to the same drinking water concentrations of sodium dichromate dihydrate as "core" animals for up to 53 weeks. For the "special study" mice only, blood was collected on day 22 and at 3, 6, and 12 months for hematologic analyses only (i.e., Hct; Hgb concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet

morphology; MCV; MCH; MCHC; and leukocyte count and differentials). At the end of the 53-week treatment period, "special study" animals were evaluated for chromium tissue distribution (see Section 3.2 for the results of this study).

Survival rates of "core" study mice exposed to sodium dichromate dihydrate were similar to controls (NTP, 2008). Throughout the study, water consumption by males and females was decreased in the two highest dose groups compared with controls. During the second year of the study, water consumption in the two highest dose groups was decreased by 15 and 35%, respectively, in males and by 25 and 32%, respectively, in females (statistical significance not reported). No data on food consumption were reported. At the end of the 2-year treatment period, body weight in males in the highest dose group was decreased by 6% compared with controls (statistical significance not reported), and body weight in females in the two highest dose groups was decreased by 8 and 15%, respectively. NTP (2008) suggested that decreased body weights in the highest dose groups may have been partially due to reduced water consumption because of poor drinking water palatability, rather than an adverse effect of sodium dichromate dihydrate exposure. No treatment-related signs of clinical toxicity were observed throughout the study.

Results of hematology analyses in "special study" female mice (hematology was not assessed in male mice) showed that exposure to sodium dichromate dihydrate in drinking water produced hypochromic microcytosis (NTP, 2008), characterized by dose-related decreases in MCV and MCH and increases in erythrocyte counts (Table 4-17); the magnitude of change in other hematological parameters was small (≤5% compared with controls). The pattern of dose- and duration-related severity in female mice was similar to that observed in male "special study" rats (as described above); however, severity in mice was less than in rats. Thus, exposure of female mice to sodium dichromate dihydrate in drinking water produced microcytosis at subchronic exposure durations (22 days to 3 months), with decreased severity at 6–12 months.

Table 4-17. Hematological effects in female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for up to 12 months

Hematological parameter	Time on treatment	Treatment group (mg hexavalent chromium/kg-d)				
		0	0.38	1.4	3.1	8.7
MCV (fL)	D 22	48.8 ± 0.2[a]	48.3 ± 0.1[b] (90.0)	47.8 ± 0.2[c] (98.0)	47.0 ± 0.2[c] (96.3)	46.8 ± 0.2[c] (95.9)
	Mo 3	47.2 ± 0.1	46.9 ± 0.3 (99.4)	46.7 ± 0.1 (98.9)	45.1 ± 0.2[c] (95.6)	43.7 ± 0.3[c] (92.6)
	Mo 6	45.8 ± 0.2	45.5 ± 0.3 (99.3)	45.1 ± 0.2[b] (98.5)	44.6 ± 0.2[c] (97.4)	42.8 ± 0.3[c] (93.4)
	Mo 12	46.9 ± 0.3	46.9 ± 0.3 (100)	46.3 ± 0.3 (98.7)	45.2 ± 0.2[c] (96.4)	43.9 ± 0.5[c] (93.6)
MCH (ρg)	D 22	16.4 ± 0.1	16.2 ± 0.0[b] (98.8)	15.9 ± 0.1[c] (97.0)	15.7 ± 0.1[c] (95.7)	15.5 ± 0.1[c] (94.5)
	Mo 3	15.8 ± 0.0	15.7 ± 0.1 (99.4)	15.6 ± 0.0[c] (98.7)	14.9 ± 0.1[c] (88.6)	14.3 ± 0.1[c] (90.5)
	Mo 6	15.3 ± 0.1	15.2 ± 0.1 (99.3)	15.1 ± 0.1 (98.7)	14.9 ± 0.1[c] (97.4)	14.1 ± 0.1[c] (92.2)
	Mo 12	15.5 ± 0.1	15.7 ± 0.2 (101.3)	15.5 ± 0.1 (100)	15.1 ± 0.1[b] (97.4)	14.4 ± 0.2[c] (92.9)
Erythrocyte count (10^6/μL)	D 22	10.25 ± 0.15	10.20 ± 0.08 (99.5)	10.47 ± 0.19 (102.1)	10.77 ± 0.13[b] (105.1)	10.61 ± 0.13[b] (103.5)
	Mo 3	10.10 ± 0.16	10.66 ± 0.13[b] (105.5)	10.55 ± 0.17[b] (104.5)	10.95 ± 0.10[c] (108.4)	11.55 ± 0.16[c] (114.4)
	Mo 6	10.56 ± 0.15	10.81 ± 0.10 (102.4)	10.60 ± 0.13 (100.4)	10.77 ± 0.20 (102.0)	11.50 ± 0.20[c] (108.9)
	Mo 12	9.58 ± 0.10	9.72 ± 0.09 (101.4)	9.77 ± 0.10 (102.0)	9.95 ± 0.13[b] (103.9)	10.30 ± 0.21[c] (107.5)

[a]Values are means ± SE; values in parenthesis are percent of control; n = 10 mice/group, with the exception of 1.4 mg hexavalent chromium/kg-d group on mo 12 (n = 9).
[b]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.
[c]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.

Source: NTP (2008).

Gross and microscopic examinations of "core" study mice exposed to sodium dichromate dihydrate in drinking water for 2 years showed nonneoplastic lesions of the small intestine, liver, lymph nodes, and pancreas, and neoplastic lesions of the small intestine (NTP, 2008). Incidence data for nonneoplastic lesions are summarized in Table 4-18. In the small intestine, statistically significant increases in the incidences of minimal-to-mild diffuse epithelial hyperplasia of the duodenum were observed in male and female mice in all treatment groups and of the jejunum in females at 8.7 mg hexavalent chromium/kg-day, compared with controls. NTP (2008) noted that diffuse epithelial hyperplasia is consistent with tissue regeneration following epithelial cell damage. Incidences of minimal-to-mild histiocytic cellular infiltration of the duodenum were

increased at ≥2.4 and ≥3.1 mg hexavalent chromium/kg-day in males and females, respectively, and of the jejunum at 8.7 mg hexavalent chromium/kg-day in females, compared with controls. Moderate-to-severe focal epithelial hyperplasia was also observed in the duodenum in males and females, although incidences were not significantly different from controls (the incidence did not exceed 2/50 rats in any dose group) and did not exhibit dose-dependence. Due to its morphological similarity to adenoma, focal epithelial hyperplasia was classified as a preneoplastic lesion by NTP (2008).

Table 4-18. Incidence of nonneoplastic lesions observed in male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

Tissue (lesion type)	Treatment group (mg hexavalent chromium/kg-d)				
	0	**0.38**	**0.91**	**2.4**	**5.9**
Males					
Small intestine, duodenum (diffuse epithelial hyperplasia)	0/50[a]	11/50[b] (2.0)	18/50[b] (1.6)	42/50[b] (2.1)	32/50[b] (2.1)
Small intestine, duodenum (histiocytic cellular infiltration)	0/50	2/50 (1.0)	4/50 (1.0)	37/50[b] (1.2)	35/50[b] (1.7)
Lymph node, mesenteric (histiocytic cellular infiltration)	14/47 (1.2)	38/49[b] (1.1)	31/49[b] (1.2)	32/49[b] (1.5)	42/46[b] (2.5)
Lymph node, pancreatic (histiocytic cellular infiltration)	0/5	2/13 (1.0)	2/10 (1.0)	5/8[c] (1.4)	12/16[c] (2.3)
Pancreas (cytoplasmic alteration)	0/49	1/49 (3.0)	1/50 (3.0)	9/49[b] (2.1)	8/48[b] (2.6)
Tissue (lesion type)	**Treatment group (mg hexavalent chromium/kg-d)**				
	0	**0.38**	**1.4**	**3.1**	**8.7**
Females					
Small intestine, duodenum (diffuse epithelial hyperplasia)	0/50[a]	16/50[b] (1.6)	35/50[b] (1.7)	31/50[b] (1.6)	42/50[b] (2.2)
Small intestine, duodenum (histiocytic cellular infiltration)	0/50	0/50	4/50 (1.3)	33/50[b] (1.2)	40/50[b] (2.0)
Small intestine, jejunum (diffuse epithelial hyperplasia)	0/50	2/50 (2.0)	1/50 (1.0)	0/50	8/50[b] (1.9)
Small intestine, jejunum (histiocytic cellular infiltration)	0/50	0/50	0/50	2/50 (1.0)	8/50[b] (1.6)
Liver (histiocytic cellular infiltration)	2/49 (1.0)	15/50[b] (1.1)	23/50[b] (1.0)	32/50[b] (1.0)	45/50[b] (1.9)
Liver (chronic inflammation)	16/49 (1.1)	21/50 (1.1)	22/50 (1.10	27/50[b] (1.1)	24/50 (1.0)
Lymph node, mesenteric (histiocytic cellular infiltration)	3/46 (1.0)	29/48[b] (1.3)	26/46[b] (1.1)	40/50[b] (1.9)	42/50[b] (2.7)
Lymph node, pancreatic (histiocytic cellular infiltration)	0/14	1/12 (1.0)	2/15 (1.5)	7/14[b] (1.9)	8/13[b] (2.5)
Pancreas (cytoplasmic alteration)	0/48	6/50[c] (2.5)	6/49[c] (2.0)	14/50[b] (2.4)	32/50[b] (2.6)

[a]Number of animals with lesion/number of animals examined; parenthesis indicate average severity grade, with 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[b]Significantly different ($p \leq 0.01$) from the control group by the Poly-3 test.
[c]Significantly different ($p \leq 0.05$) from the control group by the Poly-3 test.

Source: NTP (2008).

In the liver of female mice, dose-dependent increases were observed in the incidences of histiocytic infiltration at all doses and of chronic inflammation in the 3.1 mg hexavalent

chromium/kg-day group, both lesions were minimal to mild in severity. Significant decreases in the incidences of clear cell and eosinophilic foci were observed in the liver of males at 5.9 mg hexavalent chromium/kg-day and of eosinophilic foci in the liver of females at ≥3.1 mg hexavalent chromium/kg-day; NTP (2008) indicated that the biological significance of these decreases is uncertain.

Dose-dependent increases in the incidences and severity (minimal-to-mild) of histiocytic cellular infiltration of the mesenteric lymph nodes were observed in males and females in all treatment groups and of the pancreatic lymph nodes in males and females at ≥2.4 and ≥3.1 mg hexavalent chromium/kg-day, respectively, compared with controls.

In the pancreas, the dose-dependent increases in the incidences and severity (mild-to-moderate) of cytoplasm alterations, characterized by depletion of cytoplasm zymogen granules, were observed at ≥2.4 mg hexavalent chromium/kg-day in males and in all treatment groups in females. NTP (2008) stated that the biological significance of this finding is uncertain.

Incidence data for neoplastic lesions of the small intestine in male and female mice exposed to sodium dichromate dihydrate in drinking water for 2 years are summarized in Table 4-19 (NTP, 2008). In male mice, incidences of combined small intestine (duodenum, jejunum, and ileum) adenoma or carcinoma were significantly increased at ≥2.4 mg hexavalent chromium/kg-day and incidences of duodenal adenoma, small intestine adenoma, and small intestine carcinoma were significantly increased at 5.9 mg hexavalent chromium/kg-day. In addition, significant positive dose-related trends were observed for the incidences of duodenal adenoma, duodenal carcinoma, jejunal adenoma, small intestine adenoma, small intestine carcinoma, and combined small intestine adenoma or carcinoma. In female mice, significant increases in the incidences of duodenal adenoma, small intestine adenoma, and combined small intestine adenoma or carcinoma were observed at ≥3.1 mg hexavalent chromium/kg-day and incidences of duodenal carcinoma, jejunal adenoma, and small intestine carcinoma were significantly increased at 8.7 mg hexavalent chromium/kg-day. Significant positive dose-related trends were observed for duodenal adenoma, duodenal carcinoma, jejunal adenoma, small intestine adenoma, small intestine carcinoma, and combined small intestine adenoma or carcinoma. No other statistically or biologically significant neoplasms were observed in other tissues.

Table 4-19. Incidence of neoplastic lesions observed in the small intestine of male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

Tissue and lesion type	Treatment group (mg hexavalent chromium/kg-d)				
	0	0.38	0.91	2.4	5.9
Males					
Duodenum, adenoma					
Overall rate[a,b]	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	6/50 (12%) $p \leq 0.05$
Duodenum, all adenoma (includes multiple adenomas)					
Overall rate[a]	1/50 (2%) [665]	0/50 (0%)	1/50 (2%) [729T]	5/50 (10%) [729T]	15/50 (30%) [451]
Adjusted rate[c]	2.2% $p < 0.001$	0%	2.3%	10.8%	32.9% $p < 0.001$
Duodenum, carcinoma					
Overall rate[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%) [729T]	3/50 (6%) [729T]
Adjusted rate[c]	0% $p < 0.011$	0%	0%	4.3%	6.8%
Jejunum, adenoma					
Overall rate[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%) [714]
Adjusted rate[c]	0% $p = 0.002$	0%	0%	0%	6.8%
Jejunum, multiple carcinoma					
Overall rate[a,b]	0/50	1/50	0/50	0/50	0/50
Jejunum, all carcinoma (includes multiple)					
Overall rate[a,b]	0/50	2/50	0/50	1/50	2/50
All small intestine[d], adenoma					
Overall rate[a]	1/50 (2%) [665]	1/50 (2%) [729T]	1/50 (2%) [729T]	5/50 (10%) [729T]	17/50 (34%) [451]
Adjusted rate[c]	2.2% $p < 0.001$	2.3%	2.3%	10.8%	37.2% $p < 0.001$
All small intestine[d], carcinoma					
Overall rate[a]	0/50 (0%)	2/50 (4%) [729T]	1/50 (2%) [729T]	3/50 (6%) [729T]	5/50 (10%) [729T]
Adjusted rate[c]	0% $p = 0.014$	4.5%	2.3%	6.5%	11.4% $p = 0.028$
All small intestine[d], adenoma or carcinoma					
Overall rate[a]	1/50 (2%) [665]	3/50 (6%) [729T]	2/50 (4%) [729T]	7/50 (14%) [729T]	20/50 (40%) [451]
Adjusted rate[c]	2.2% $p < 0.001$	6.8%	4.6%	15.1% $p = 0.032$	43.8% $p < 0.001$

Table 4-19. Incidence of neoplastic lesions observed in the small intestine of male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

Tissue and lesion type	Treatment group (mg hexavalent chromium/kg-d)				
	0	0.38	1.4	3.1	8.7
Females					
Duodenum, multiple adenoma					
Overall rate[a,b]	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	6/50 (12%) $p \leq 0.05$
Duodenum, all adenoma (includes multiple)					
Overall rate[a]	0/50 (0%)	0/50 (0%)	2/50 (4%) [729T]	13/50 (25%) [729T]	12/50 (24%) [693]
Adjusted rate[c]	0% $p < 0.001$	0%	4.2%	27.8% $p < 0.001$	25.2% $p < 0.001$
Duodenum, carcinoma					
Overall rate[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%) [729T]	6/50 (12%) [625]
Adjusted rate[c]	0% $p < 0.001$	0%	0%	2.1%	12.6% $p = 0.019$
Jejunum, multiple adenomas					
Overall rate[a,b]	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Jejunum, all adenomas (including multiple)					
Overall rate[a]	0/50 (0%)	1/50 (2%) [729T]	0/50 (0%)	2/50 (4%) [729T]	5/50 (10%) [729T]
Adjusted rate[c]	0% $p = 0.002$	2.2%	0%	4.3%	10.6% $p = 0.035$
Jejunum, carcinoma					
Overall rate[a,b]	1/50 (2%)	0/50 (0%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
All small intestine[d], adenoma					
Overall rate[a]	0/50 (0%)	1/50 (2%) [729T]	2/50 (4%) [729T]	15/50 (30%) [729T]	16/50 (32%) [693]
Adjusted rate[c]	0% $p < 0.001$	2.2%	4.2%	32.0% $p < 0.001$	33.7% $p < 0.001$
All small intestine[d], carcinoma					
Overall rate[a]	1/50 (2%) [729T]	0/50 (0%)	2/50 (4%) [729T]	3/50 (6%) [729T]	7/50 (14%) [625]
Adjusted rate[c]	2.2% $p < 0.001$	0%	4.2%	6.4%	14.7% $p = 0.037$

Table 4-19. Incidence of neoplastic lesions observed in the small intestine of male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

All small intestine[d], adenoma or carcinoma					
Overall rate[a]	1/50 (2%) [729T]	1/50 (2%) [729T]	4/50 (8%) [729T]	17/50 (34%) [729T]	22/50 (44%) [625]
Adjusted rate[c]	2.2% $p < 0.001$	2.2%	8.3%	36.3% $p < 0.001$	45.9% $p < 0.001$

[a]Overall rate: number of animals with lesion/number of animals examined; parentheses are the percent of animals examined with lesion; brackets indicate the days to first incidence; T: observed at terminal sacrifice. *p*-Value under treatment group incidence data indicates statistically significant Poly-3 test for pairwise comparison between control and exposed group. Statistical analysis using overall rates were only conducted if adjusted rates were not determined.
[b]Adjusted rate not reported.
[c]Adjusted rate: Poly-3 estimated neoplasm incidence (expressed as % of animals with neoplasm) adjusted for intercurrent mortality. *p*-Value under control group indicates statistically significant positive Poly-3 trend test. *p*-Value under treatment group incidence data indicates statistically significant Poly-3 test for pairwise comparison between control and exposed groups, using adjusted rates.
[d]Duodenum, jejunum, or ileum.

Source: NTP (2008).

In conclusion, from the NTP (2008) 2-year toxicology and carcinogenicity study on sodium dichromate dihydrate, EPA identified a LOAEL for noncancer effects of 0.38 mg hexavalent chromium/kg-day in male and female B6C3F$_1$ mice; a NOAEL value was not identified because effects were seen at the lowest dose administered. In males, the LOAEL was based on increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia) and mesenteric lymph nodes (histiocytic cellular infiltration); in females, the LOAEL was based on increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia), mesenteric lymph nodes (histiocytic cellular infiltration), liver (histiocytic cellular infiltration), and pancreas (depletion of cytoplasmic zymogen granules). Although mild microcytic, hypochromic anemia was observed in female mice at ≥0.38 mg hexavalent chromium/kg-day after 22 days of exposure, the severity of these effects decreased over time, such that only small changes (<5%) were observed at ≥3.1 mg hexavalent chromium/kg-day after 12 months of exposure; therefore, hematological effects were not considered by EPA as the basis for the chronic LOAEL value in female mice. In addition to noncancer effects, exposure of B6C3F$_1$ mice to sodium dichromate dihydrate in drinking water for 2 years resulted in significant increases in the incidences of neoplasms of the small intestine in males and females at doses ≥2.4 and ≥3.1 mg hexavalent chromium/kg-day, respectively. NTP (2008) concluded that results of this study provide clear evidence of carcinogenic activity of sodium dichromate dihydrate in male and female B6C3F$_1$ mice based on increased incidences of neoplasms of the small intestine.

Although water consumption was reduced in both male and female rats and mice at the two highest doses in the NTP (2008) study, the NTP concluded that the animals in this two-year bioassay were not suffering from dehydration, and thus this reduced water consumption had little impact on the study results. More specifically, the NTP stated the following regarding this potential dehydration issue in their final Technical Report (NTP, 2008):

> The lower body weights observed in male and female rats and mice exposed to the two highest exposure concentrations were partly attributed to poor palatability of the dosed water and consequent reductions in water consumption. However, several lines of evidence suggest that the animals were not dehydrated. When water consumption is adjusted for body weight (data not shown), dosed male and female rats and female mice drank approximately the same quantities of water per gram of body weight as the controls after the first 20 weeks on study. Male mice exposed to 257.4 mg/L drank less water per gram of body weight than did the controls throughout the study. Although mean body weights and water consumption were reduced in the higher exposure concentration groups, the average daily doses (mg sodium dichromate dihydrate per kilogram body weight) were in the same proportions as the drinking water concentrations (mg/L) for male and female rats and mice. Clinical observations related to dehydration including loss of skin turgor, dry mucous membranes, retraction of eyes, hypoactivity, and poor hair coats were not observed in rats or mice in the 2-year studies of sodium dichromate dihydrate. Abnormalities in hematology and clinical chemistry parameters that typically indicate dehydration include increases in hematocrit, urine specific gravity, and serum concentrations of albumin, total protein, and urea nitrogen. In the current 2-year studies, hematology and clinical chemistry parameters were measured in male rats on days 4 and 22 and at months 3, 6, and 12. Significant decreases in hematocrit and serum concentrations of albumin and total protein were observed in males exposed to 516 mg/L. Taken together, these data suggest that the neoplastic and nonneoplastic effects of sodium dichromate dihydrate were not associated with dehydration.

Borneff et al., 1968

Borneff et al. (1968) conducted a long-term animal cancer bioassay of hexavalent chromium administered in drinking water. Using a three-generation study design, Borneff et al. (1968) treated 120 female and 10 male NMRI mice with 1 mg potassium chromate/day (500 ppm) in drinking water (containing 3% household detergent). A control group of animals received drinking water (3% detergent) only. An outbreak of mousepox (ectromelia) virus occurred during the 8th month of the experiment, and within 3 months, the majority (512) of the animals died. All animals received a mousepox vaccination 2 months after the outbreak, and this effectively ended the epidemic and the study continued. Two carcinomas of the stomach were observed in female mice exposed to potassium chromate. No malignant stomach tumors were found in control mice. Nine benign stomach tumors were observed in female mice exposed to potassium chromate. The combined incidence of malignant and benign stomach tumors (11/66)

in potassium chromate-exposed-female mice was significantly different than the combined incidence of tumors in control female mice (2/79; Fisher's exact test, $p < 0.05$). This increase in tumors, however, was seen almost exclusively in the generation most affected by the epidemic, and it is likely that the observed increase in tumors was due, at least in part, to the infection. Because of the unknown impact of the mousepox infection on the results of this study, as well as other methodological problems, EPA chose not to identify a NOAEL or LOAEL from this study. Furthermore, this study is considered unsuitable for the assessment of the oral carcinogenicity of hexavalent chromium.

Anwar et al., 1961

The effects of chronic oral exposure to hexavalent chromium were evaluated in dogs by Anwar et al. (1961). Dogs (one control dog and one to two dogs/treatment group) were exposed to potassium chromate in drinking water at concentrations of 0, 0.45, 2.25, 4.5, 6.75, or 11.2 mg hexavalent chromium/L for 4 years. Several different breeds of dogs (German shepherds, poodles, and beagles) were used and body weights of animals were not reported; thus, daily hexavalent chromium doses cannot be accurately estimated. Throughout the exposure period, animals were evaluated for clinical signs of toxicity, and food consumption and growth rate were recorded (frequency of observations not reported). At monthly intervals, blood was obtained for evaluation of hematology (i.e., erythrocyte counts, total and differential leukocyte counts, and Hgb), and at 6-month intervals, urine was analyzed for albumin, acetone, bile pigments, glucose, erythrocytes, and specific gravity. At the end of the 4-year treatment period, weights of the liver, kidney, and spleen were recorded, and microscopic examination was conducted on selected tissues of major organs. No chromium-related effects were observed. Interpretation of the study results is limited by the small number of animals evaluated and the inability to estimate daily doses of hexavalent chromium received by the treated animals. A NOAEL or LOAEL could not be identified from this study by EPA.

MacKenzie et al., 1958

MacKenzie et al. (1958) conducted two experiments in which Sprague-Dawley rats were administered hexavalent chromium in drinking water for 1 year. In the first experiment, groups of rats (10 per sex in the control group and 8 per sex in the treatment groups) were exposed to drinking water containing potassium chromate at concentrations of 0, 0.45, 2.2, 4.5, 7.7, or 11 mg hexavalent chromium/L. In the second experiment, groups of 12 male and 9 female rats were exposed to drinking water containing potassium chromate at concentrations of 0 or 25 mg hexavalent chromium/L. For experiment 1, MacKenzie et al. (1958) reported that drinking water consumption and body weights in the treatment groups were comparable to controls, although data were not reported. Using reference values for body weight (males: 0.523 kg; females: 0.338 kg) and daily drinking water intake (males: 0.062 L/day; females: 0.045 L/day) for adult

male and female Sprague-Dawley rats (U.S. EPA, 1988), doses of 0.05, 0.26, 0.53, 0.91, or 1.3 mg hexavalent chromium/kg-day for males and 0.06, 0.29, 0.60, 1.0, or 1.5 mg hexavalent chromium/kg-day for females exposed to drinking water containing 0.45, 2.2, 4.5, 7.7, or 11 mg hexavalent chromium/L, respectively, were estimated by EPA. For experiment 2, drinking water consumption was decreased by 16 and 27% in male and female rats, respectively. Thus, using reference values for body weight and daily drinking water intake for adult male and female Sprague-Dawley rats (listed above; U.S. EPA, 1988) and assuming decreases in water consumption of 16 and 27% in males and females, respectively, average daily doses of 2.8 and 2.4 mg hexavalent chromium/kg-day in males and females, respectively, were estimated by EPA. Throughout the treatment period in both experiments, animals were examined for clinical signs of toxicity, and weight gain and food and water consumption were recorded (frequency of observations not reported). At monthly intervals, blood was analyzed for Hgb, erythrocyte counts, and total and differential leukocyte counts. At the end of treatment, microscopic examinations of selected tissues (kidney, adrenal gland, liver, spleen, heart, brain, stomach, duodenum, ileum, colon, and bone marrow) were conducted (as described by Decker et al., 1958). No treatment-related clinical signs of toxicity, effects on food consumption, body weight gain, or histopathological findings were observed. Therefore, EPA identified a NOAEL of 2.8 mg/kg-day.

4.3. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES—ORAL

Studies evaluating the potential reproductive effects of oral exposure to hexavalent chromium compounds have been conducted in monkeys (Aruldhas et al., 2006, 2005, 2004; Subramanian et al., 2006), rats (Bataineh et al., 2007, 1997; Elsaieed and Nada, 2002; Li et al., 2001; Kanojia et al., 1998, 1996; NTP, 1996b; Chowdhury and Mitra, 1995;), mice (Al-Hamood et al., 1998; Elbetieha and Al-Hamood, 1997; NTP, 1997; 1996a; Junaid et al., 1996a, b, 1995; Murthy et al., 1996; Zahid et al., 1990; Trivedi et al., 1989), and rabbits (Yousef et al., 2006). In addition, several studies have specifically evaluated the potential effects of pregestational, gestational, or lactational exposure on fetal development in rats (Banu et al., 2008; Elsaieed and Nada, 2002; Kanojia et al., 1998, 1996) and mice (Al-Hamood et al., 1998; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989). Studies conducted by NTP (1997, 1996a,b) and Zahid et al. (1990) evaluated dietary exposure; all other studies evaluated animals exposed to hexavalent chromium in drinking water or by gavage. In general, studies that evaluated developmental effects of hexavalent chromium were conducted at higher exposure levels than those that evaluated reproductive effects.

Collectively, the available studies provide evidence that oral exposure of laboratory animals to hexavalent chromium compounds produces adverse reproductive effects, including histopathological changes to reproductive organs in males (Aruldhas et al., 2006, 2005, 2004; Chowdhury and Mitra, 1995; Li et al., 2001; Zahid et al., 1990) and females (Murthy et al.,

1996); alterations in sperm, including decreased count, decreased motility, and abnormal morphology (Subramanian et al., 2006; Yousef et al., 2006; Li et al., 2001; Zahid et al., 1990); decreased plasma testosterone levels (Yousef et al., 2006; Chowdhury and Mitra, 1995); increased estrous cycle length (Kanojia et al., 1998, 1996; Murthy et al., 1996); changes in mating behavior and decreased fertility in males (Bataineh et al., 1997); and adverse reproductive outcomes, including decreased numbers of live fetuses and implantations, and increased numbers of resorptions and pre- and postimplantation losses (Bataineh et al., 2007; Elsaieed and Nada, 2002; Elbetieha and Al-Hamood, 1997; Junaid et al., 1996a,b, 1995; Kanojia et al., 1998, 1996; Trivedi et al., 1989). Developmental effects observed have included decreased fetal weight and length (Elsaieed and Nada, 2002; Kanojia et al., 1998; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989); external (subdermal hemorrhage and tail malformations) and skeletal abnormalities (decreased ossification) (Elsaieed and Nada, 2002; Junaid et al., 1996a, b, 1995; Kanojia et al., 1998, 1996; Trivedi et al., 1989); and delayed sexual maturation and function in female offspring (Banu et al., 2008; Al-Hamood et al., 1998). In contrast to results of the above studies, adverse effects were not observed in dietary exposure studies conducted by NTP that investigated the potential for hexavalent chromium to produce adverse effects on male reproductive organs in rats and mice (NTP, 1996a,b) and on reproductive outcomes in a continuous breeding study in mice (NTP, 1997).

The following review of available reproductive and developmental studies is organized as follows: (1) studies evaluating effects on reproductive tissues and mating behavior, (2) studies evaluating effects on reproductive outcomes, (3) studies evaluating pregestational exposure on reproductive outcomes and fetal development, and (4) studies evaluating gestational and/or lactational exposure on reproductive outcomes and fetal development. The results of these studies are summarized in Section 4.5, Synthesis of Major Noncancer Effects, Table 4-26.

4.3.1. Effects on Reproductive Tissues and Mating Behavior

Aruldhas et al., 2006, 2005, 2004; Subramanian et al., 2006

In a series of studies conducted by the same research group, adverse effects on male reproductive organs were observed in monkeys exposed to hexavalent chromium in drinking water (Aruldhas et al., 2006, 2005, 2004; Subramanian et al., 2006). All of these studies followed the same exposure protocol; adult male bonnet monkeys (6–8 years old) were exposed to drinking water containing 0, 100, 200, or 400 mg potassium dichromate/L in Aruldhas et al. (2006, 2005, 2004) or 0, 50, 100, 200, or 400 mg potassium dichromate/L in Subramanian et al. (2006) for 180 days; two of the studies included a 180-day post-treatment recovery period (Aruldhas et al., 2006; Subramanian et al., 2006). Aruldhas et al. (2004) noted that 400 mg potassium dichromate/L was selected as the maximum concentration tested because exposure to higher concentrations resulted in decreased food and drinking water consumption and death within 3 months. At the beginning of the treatment period, body weights of monkeys were

reported as 7–8 kg by Aruldhas et al. (2005) and 7–9 kg by Subramanian et al. (2006). Although body weights were not reported by Aruldhas et al. (2006, 2004), it is assumed that initial body weights were similar in all studies. The study authors did not report body weights or drinking water consumption over the course of treatment or calculate daily doses of hexavalent chromium. For this review, daily doses of 0, 1.0, 2.1, 4.1, and 8.3 mg hexavalent chromium/kg-day for the 0, 50, 100, 200, or 400 potassium dichromate/L groups, respectively, were estimated using the allometric equation for drinking water consumption for primates ($0.09 \times \text{body weight}^{0.7945}$; U.S. EPA, 1988) and an average reported initial body weight of 8 kg (Subramanian et al., 2006; Aruldhas et al., 2005); however, these dose estimates are uncertain due to the absence of data on body weight and drinking water consumption over the course of the 6-month treatment period. In the following discussions, the three treatment groups evaluated in the Aruldhas et al. (2006, 2005, 2004) studies (i.e., 100, 200, and 400 mg potassium dichromate/L, approximately equivalent to 2.1, 4.1, and 8.3 mg hexavalent chromium/kg-day, respectively) are referred to as the low-, mid-, and high-dose groups, respectively; the four treatment groups evaluated in the Subramanian et al. (2006) study (i.e., 50, 100, 200, and 400 mg potassium dichromate/L, approximately equivalent to 1.0, 2.1, 4.1, and 8.3 mg hexavalent chromium/kg-day, respectively) are referred to as the lowest-, low-, mid-, and high-dose groups, respectively.

Aruldhas et al. (2004) conducted histological assessments of testes and epididymides from monkeys (three monkeys/group) following 180 days of treatment. Testes and epididymides were evaluated by light microscopy (resin-embedded slices) and transmission electron microscopy (TEM). In the three treatment groups, epididymal damage and the development of microcanals in the cauda epididymal epithelium were observed; severity of ductal damage increased with dose. In the low-dose group, the cauda epididymal epithelium appeared pseudostratified; degeneration of principal cells and epithelial rupture, with the lumen occluded by principal cells, were observed. In the mid-dose group, the occluded lumen appeared packed with immature germ cells and macrophages. In the high-dose group, hypertrophy of the caudal epithelium and "obliteration" of the ductal lumen were observed. The development of two morphologically distinct microcanals was observed in all treatment groups. Arulhhas et al. (2004) proposed that microcanal development was an adaptive response to provide passage for spermatozoa around the obstructed ducts and to entrap spermatozoa that had been released into the epithelium due to the epithelial rupture. Appearance of tissues from the control group was not reported. Additional TEM evaluations of testes from monkeys (three monkeys/group) in the three hexavalent chromium treatment groups showed a dose-related accumulation of basal cells along the basal lamina of the epididymis, giving the epithelium a pseudostratified appearance, and intraepithelial macrophages (Aruldhas et al., 2006). In addition, cells showed an accumulation of sperm-derived lipofuscin material, indicative of phagocytosis and processing of sperm. In contrast, these findings were not observed in testes from control monkeys.

Aruldhas et al. (2005) evaluated the effects of hexavalent chromium exposure in male monkeys at the completion of the 180-day treatment period (three monkeys/group) and following an additional 180-day recovery period (three monkeys/group); assessments included plasma chromium concentration, absolute and relative testicular weights, and microscopic (light and TEM) evaluations of testes. At the end of the treatment period, chromium plasma concentration was significantly ($p < 0.05$) increased in the three treatment groups, with increases reaching almost ninefold in the high-dose group compared to controls. Relative testicular weight was significantly ($p < 0.05$) decreased by 23, 35, and 34% in the low-, mid-, and high-dose groups, respectively; absolute testicular weight was not affected by treatment (data not reported). Following the recovery period, chromium plasma concentrations and relative testicular weight in treatment groups were comparable to controls. Light microscopic evaluations of testes in control monkeys showed seminiferous tubules and Leydig cells with normal appearance and cellular organization. In the three hexavalent chromium treatment groups, seminiferous tubules appeared disorganized, with decreased diameters, epithelial degeneration, and lumens filled with prematurely released germ cells and cellular debris; depletion of germ cells, hyperplasia of Leydig cells, and Sertoli cell fibrosis were also observed. TEM examination of testes from the three treatment groups showed morphological changes in spermatids (granulation of chromatin and vacuolization) and spermatocytes (fragmented chromatin and swollen mitochondria) and the presence of macrophages containing phagocytosed sperm; effects were more severe in the high-dose group. Following the recovery period, no histopathological findings were observed in testes of hexavalent chromium-treated monkeys, with the exception of "a few" prematurely released germ cells in the seminiferous tubular lumen (treatment group for this observations was not specified).

Subramanian et al. (2006) evaluated sperm count and sperm straight-line velocity at monthly intervals during the 180-day treatment period; the same evaluations were conducted monthly in monkeys in the high-dose group during a 180-day recovery period. In the lowest-dose group, no effects were observed on sperm count or straight-line velocity. Sperm count was significantly decreased in the low-, mid-, and high-dose groups, compared with controls; decreases were dose- and duration-dependent. For example, in the low-dose group, significant ($p < 0.05$) decreases in sperm count were first observed after 4 months (11% decrease), with a maximum decrease of 25% after 6 months; in the high-dose group, sperm counts were significantly decreased by 13% after 2 months, with a 30% reduction after 6 months. Similar effects were observed for sperm straight-line velocity. In the low-dose group, velocity was significantly ($p < 0.05$) decreased by 10 and 25% after 4 and 6 months of treatment, respectively; in the high-dose group, velocity was significantly decreased by 12% after 2 months and by 35% after 6 months. Effects on sperm count and straight-line velocity were reversible following withdrawal from treatment. During the first month of the recovery period (high-dose monkeys only), sperm count was significantly increased compared with that observed at the end

of the treatment period, with counts returning to pretreatment levels by month 3 of the recovery period; sperm velocity returned to pretreatment levels by month 3 of the recovery period.

Results of these four studies (Aruldhas et al., 2006, 2005, 2004; Subramanian et al., 2006) indicate that exposure of monkeys to hexavalent chromium as potassium dichromate in drinking water produced reversible changes to male reproductive organs, including disruption of spermatogenesis. Effects on sperm count and velocity and histopathological changes were observed in the low-, mid-, and high-dose groups (≥2.1 mg hexavalent chromium/kg-day), but no effects on sperm count or velocity were observed in monkeys in the lowest treatment group (1 mg hexavalent chromium/kg-day). This dose cannot be considered a NOAEL, however, because microscopic evaluations were not conducted in monkeys from this group. For this reason, NOAEL and LOAEL values from these studies were not identified by EPA. Although group sizes in these studies were small, the results provide evidence of adverse male reproductive effects in nonhuman primates exposed to hexavalent chromium in drinking water at concentrations as low as 35.3 mg hexavalent chromium/L (2.1 mg hexavalent chromium/kg-day).

Chowdhury and Mitra, 1995

Effects of oral exposure to hexavalent chromium on male reproductive organs was evaluated in mature (age not reported) male Charles Foster rats that were administered 0, 20, 40, or 60 mg hexavalent chromium/kg-day as sodium dichromate in saline by gavage for 90 days (Chowdhury and Mitra, 1995). Although Chowdhury and Mitra (1995) stated that the control and exposure groups included 10 animals per group, conflicting summaries of the actual group sizes are presented in the report. Body weights were recorded twice weekly. At the end of the treatment period, testes were excised, weighed, and prepared for histological or biochemical evaluations, and serum testosterone activity was determined. For biochemical analyses, fresh tissue was homogenized and assayed for total cholesterol, activities of succinic dehydrogenase and 3β-Δ^5-hydroxysteroid dehydrogenase (3β-Δ^5-HSH), and total protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). For microscopic evaluations, testes were fixed in Bouin's fluid, embedded in paraffin, and stained with haematoxylin and eosin (H&E).

Final body weight was significantly reduced by approximately 27% compared to controls in the mid- and high-dose groups (statistical significance not reported); absolute testis weights were significantly reduced by 28% ($p < 0.05$) and 35% ($p < 0.001$) in the mid- and high-dose groups, respectively, compared with controls. Serum testosterone levels were decreased by 31% in the low- ($p < 0.05$) and mid-dose ($p < 0.001$) groups and by 47% ($p < 0.001$) in the high-dose group. Biochemical analysis of testes showed significant decreases in total cholesterol by 2% ($p < 0.05$) and 25% ($p < 0.001$) in the mid- and high-dose groups, respectively, and significant ($p < 0.001$) decreases in succinic dehydrogenase activity by 35 and 45% in the mid- and high-dose groups, respectively. In all treatment groups, 3β-Δ^5-HSH was significantly decreased by

25% ($p < 0.05$), 28% ($p < 0.05$), and 52% ($p < 0.001$) in the low-, mid-, and high-dose groups, respectively. Dose-related decreases in total testicular protein were observed, with decreases reaching 46% ($p < 0.001$) in the high-dose group. Testicular DNA and RNA levels were significantly decreased in the mid- and high-dose groups, with decreases reaching 45% ($p < 0.001$) and 37% ($p < 0.001$), respectively, in the high-dose group. Microscopic evaluation of testicular tissue showed adverse effects in the mid- and high-dose groups including disintegration of peritubular membranes, detachment of seminiferous cellular components from basement membranes, and accumulation of cellular debris in the mid-dose group, and cellular degeneration and complete disruption of the epithelium with fibrous tissue in the high-dose group; reduction in seminiferous tubular diameter, decreased number of Leydig cells, and Leydig cell degeneration were observed in the mid- and high-dose groups. No change in the number of spermatogonia were observed, although the number of pachytene spermatocytes and stage 7 spermatids were decreased in the mid- and high-dose groups and resting spermatocytes were decreased in the high-dose group. No treatment-related histopathological effects were observed in the testes of rats in the low-dose group, although histochemical evaluations of testes showed dose-related loss of 3β-Δ^5-HSH activity in all treatment groups.

Results of histological and biochemical analyses show that oral exposure of male rats to hexavalent chromium for 90 days produced adverse effects on male reproductive tissues, including decreased spermatogenic and steroidogenic activities. Based on decreased serum testosterone levels and loss of 3β-Δ^5-HSH activity in testes observed in all treatment groups, EPA identified a LOAEL of 20 mg hexavalent chromium/kg-day from this gavage study of male Charles Foster rats,.

Bataineh et al., 1997

Effects of oral hexavalent chromium administration on mating behavior, aggression, and fertility were assessed in male rats by Bataineh et al. (1997). Adult (age not specified) male Sprague-Dawley rats (n = 12 or 13) were administered drinking water containing 0 or 1,000 mg potassium dichromate/L (equivalent to 353 mg hexavalent chromium/L) for 12 weeks. No data on drinking water consumption were included in the study report. Based on findings of other studies (NTP, 2008, 2007) showing decreased drinking water consumption and body weight at drinking water concentrations ≥30 mg hexavalent chromium/L, it is likely that drinking water consumption was decreased in the chromium treatment group; thus, daily doses of hexavalent chromium cannot be accurately estimated from this study. Following the treatment period, assessments were conducted for sexual behavior in the presence of females in estrous (number of mounts without penile intromission, time to first mount, time from presentation of female to first intromission, number of penile intromissions, time from first intromission to ejaculation, and time from ejaculation to next intromission), aggressive behavior in the presence of a second untreated male (number of lacerations given, boxing bouts, fights, and ventral presenting),

fertility following a 10-day mating period with untreated females (numbers of pregnant females, viable fetuses, and resorptions), body weight, and weights of reproductive organs (paired testes, seminal vesicles, and preputial glands). Histopathological evaluations of tissues were not conducted.

All rats "appeared healthy" throughout the treatment period. Assessment of mating behavior in hexavalent chromium-treated rats showed significant decreases in number of mounts (35% decrease; $p < 0.001$) and percentage of males ejaculating (79% decreases; $p < 0.005$), and increases in the time from first intromission to ejaculation (59% increase; $p < 0.001$) and time from ejaculation to next intromission (37% increases, $p < 0.001$), compared with controls. All measures of aggressive behavior were decreased in rats treated with potassium dichromate. All measures of fertility were comparable between control and treatment groups. Treatment resulted in significant ($p < 0.001$) decreases in body weight (19% decrease) and absolute weights of testes (24% decrease), seminal vesicles (15% decrease), and preputial gland (23% decrease); however, for relative weights of reproductive tissues, only relative testes weight was significantly decreased (6% decrease, $p < 0.05$) compared to controls.

EPA identified a LOAEL of 353 mg hexavalent chromium/L as potassium dichromate in drinking water based on adverse effects on mating and aggressive behaviors; a NOAEL was not identified because effects were observed at the only dose tested. Because drinking water consumption and body weight data over the course of the study were not provided by the investigators, a LOAEL, expressed in mg hexavalent chromium/kg-day, could not be derived from this study.

Li et al., 2001

Oral exposure of male rats to chromium(VI) oxide for 6 days resulted in adverse reproductive effects, including reduced epididymal sperm counts and increased abnormal sperm (Li et al., 2001). Groups of 8–11 male Wistar rats (60 days old) were administered chromium(VI) oxide by gavage at doses of 0, 10, or 20 mg chromium(VI) oxide/kg-day (equivalent to 0, 5.2, or 10.4 mg hexavalent chromium/kg-day, respectively) for 6 days. After 6 weeks, rats were sacrificed; testes and epididymis were removed and analyzed for epididymal sperm count and abnormal sperm; and testes were prepared (fixed in formaldehyde, embedded in paraffin, sliced, and stained with H&E) for histological evaluations of morphological abnormalities and diameter of seminiferous tubules. Epididymal sperm counts were significantly ($p < 0.05$) decreased by 76 and 80%, and the percentage of abnormal sperm was significantly ($p < 0.01$) increased by 143 and 176% in the 5.2 and 10.4 mg hexavalent chromium/kg-day groups, respectively. Treatment-related histopathological findings included decreased diameter of seminiferous tubules and disruption of germ cell arrangement within seminiferous tubules in both treatment groups. Based on decreased sperm counts and histopathological changes to the testes, 5.2 mg hexavalent chromium/kg-day was identified by EPA as a LOAEL for male rats

exposed to gavage doses of chromium(VI) oxide for 6 days; a NOAEL was not identified because effects were seen at the lowest dose administered.

Zahid et al., 1990

Zahid et al. (1990) reported adverse effects on the male reproductive system in mice fed diets containing potassium dichromate. However, other research groups (NTP 1997, 1996a, b; Finley et al., 1993) have questioned the validity of the Zahid et al. (1990) study due to concerns regarding study methods and reporting inconsistencies (as discussed below). Zahid et al. (1990) fed male weanling BALB/c albino Swiss mice diets containing 0, 100, 200, or 400 mg potassium dichromate/kg diet (equivalent to 0, 35.3, 70.6, or 141.2 mg hexavalent chromium/kg diet, respectively) for 35 days. Although Zahid et al. (1990) stated that the control and exposed groups included seven animals/group, conflicting summaries of the actual group sizes are presented throughout the report. Body weights were recorded weekly and food consumption was recorded every 48 hours. The study report stated that body weight gain and food consumption in treatment groups were comparable to the control group (data not reported); however, Zahid et al. (1990) did not calculate daily doses of hexavalent chromium. Since treatment did not affect body weight gain or food consumption, doses of 0, 6.4, 12.7, or 25.5 mg hexavalent chromium/kg-day for the 0, 35.3, 70.6, or 141.2 mg hexavalent chromium/kg diet groups, respectively, were estimated for this review using reference values for body weight (0.0316 kg) and daily food intake (0.0057 kg food/day) for subchronic exposure of male $B6C3F_1$ mice (U.S. EPA, 1988). After 35 days, testes and epididymis were weighed and then minced 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, embedded in paraffin, and subsequently sectioned to 0.6 micron thickness and stained with H&E for histological 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 either a *t*-test or a 2×2 contingency χ^2 test. Adverse effects observed in the male mouse testes included ambiguous levels of degeneration in the outermost cellular layers of the seminiferous tubules, reduced (or absent) 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. Effects were observed in all hexavalent chromium groups and the severity of effects appeared to increase with dose for percentage of degenerated tubules, percentage of tubules that were not degenerated but were without spermatogonia, percentage of abnormal sperm, and number of spermatogonia. Based on these findings, the lowest dietary concentration tested (100 mg potassium dichromate/kg diet or approximately 6.4 mg hexavalent chromium/kg-day) was identified as the LOAEL by EPA.

Other research groups (NTP, 1997, 1996a, b; Finley et al., 1993) have questioned the validity of the Zahid et al. (1990) study due to concerns regarding study design and methods. Finley et al. (1993) noted the following three concerns: (1) use of immersion fixatives (such as Bouin's fluid and paraffin embedding), which can introduce artifacts, such as grains and shrinkage, that can mimic tubular or spermatogenic pathology, (2) use of staining methods that were unable to detect the acrosome (i.e., the part of the sperm that releases enzymes to penetrate the egg) of developing spermatids, and (3) uncertainties regarding the actual groupings of animals used, the small number of animals assessed per group, and inappropriate statistical analysis of the data. NTP (1997, 1996a, b) concluded that the methods utilized by Zahid et al. (1990) were insufficient to identify spermatogonia, were likely to have 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.

Murthy et al., 1996

Effects on ovarian function were investigated in adult Swiss albino mice (90 days old; mean initial body weight of 30 g) exposed to drinking water containing potassium dichromate for 20 or 90 days (Murthy et al., 1996). For the 20-day study, groups of 30 female mice were exposed to drinking water containing 0, 250, 500, or 750 mg hexavalent chromium/L; the 20-day exposure period was selected as it coincides with one folliculogenesis cycle. For the 90-day study, groups of 10 female mice were administered drinking water containing 0, 0.05, 0.5, or 5 mg hexavalent chromium/L. The study report states that mice in both studies were evaluated daily for clinical signs of toxicity, body weight, and water and food consumption; however, no data for these outcomes were reported. Based on findings of other studies (NTP, 2008, 2007) showing decreased drinking water consumption and body weight at drinking water concentrations ≥30 mg hexavalent chromium/L, it is likely that drinking water consumption and body weight were decreased in all treatment groups in the 20-day study; thus, daily doses of hexavalent chromium cannot be accurately estimated from this study. For the 90-day study, the concentrations of hexavalent chromium in drinking water were very low and not likely to affect drinking water consumption or body weight. Thus, using reference values for body weight (0.035 kg) and daily drinking water (0.0084 L/day) intake for mature female $B6C3F_1$ mice (U.S. EPA, 1988), doses of 0, 0.01, 0.12, or 1.2 mg hexavalent chromium/kg-day were estimated by EPA for female mice exposed to drinking water containing 0, 0.05, 0.5, or 5 mg hexavalent chromium/L, respectively. In the 20-day study, three types of assessments were conducted at the end of the treatment period (each in 10 mice/group): (1) ovaries were evaluated by light microscopy and the number of follicles at each development stage, based on size (small, medium, large) and structural maturity, were determined, (2) superovulation was induced (by administration of gonadotropin) and the number of released ova were counted, and (3) estrous

cycle length was assessed (by vaginal smears) for 12 consecutive estrous cycles following treatment. In the 90-day study, all mice were sacrificed at the end of the treatment period and ovaries were evaluated by electron microscopy for ultrastructural changes.

In mice exposed for 20 days, significant ($p < 0.05$) changes in follicular development were observed in all treatment groups, with dose-related decreases in the number of small follicles in the mid- and high-dose groups and medium and large follicles in all treatment groups. In the high-dose group, the numbers of small, medium, and large follicles were reduced by 36, 53, and 72%, respectively, compared with controls. Ovarian response to gonadotropin was affected in the mid- and high-dose groups, with reductions in the number of ova released of 30 and 90%, respectively, compared with controls. Estrous cycle length was significantly increased ($p < 0.05$) by 1.7-fold in the high-dose group, compared with controls. Histopathological evaluation of ovaries after 20 days of treatment showed changes in the mid-dose (i.e., proliferated, dilated, and congested blood vessels, pyknotic nuclei in follicular cell of mature follicles) and high-dose (i.e., undeveloped follicles with degenerative cumulus cells containing dense pyknotic nuclei, neovascularization and karyorrhexis of follicular cells, erythrocytes located within stromal spaces) groups; histopathological changes were not observed in ovaries from control and low-dose mice. In mice treated for 90 days, ultrastructural changes (i.e., disintegrated cell membranes in two-layered follicular cells and alterations in mitochondria in thecal cells, which are cells of the corpus luteum that secrete estrone, estradiol, and progesterone) were observed in the high-dose group; the study report did not provide any information on ultrastructural evaluations in the low- and mid-dose groups. Murthy et al. (1996) concluded that hexavalent chromium may induce changes in ovarian function and ovulation. Due to inadequate reporting of the 20-day study (i.e., no information on effects of treatment on body weight or drinking water consumption) and the 90-day study (i.e., lack of information on ultrastructural evaluations in the low- and mid-dose groups), a LOAEL from these studies could not be identified by EPA.

Yousef et al., 2006

Adverse effects on male reproductive tissues were observed in rabbits exposed to potassium dichromate for 10 weeks (Yousef et al., 2006). Groups of six male New Zealand white rabbits (7 months old) were administered 0 or 5 mg potassium dichromate/kg-day by gavage (vehicle not specified) for 10 weeks. Yousef et al. (2006) reported that the dose of 5 mg potassium dichromate/kg-day was equivalent to 3.6 mg hexavalent chromium/kg-day. During the treatment period, food intake and body weights were recorded weekly. Semen was collected weekly and analyzed for pH and sperm count, motility, and morphology. Blood was collected every 2 weeks and analyzed for testosterone. At the end of the treatment period, animals were sacrificed and relative testes and epididymis weights were determined. At sacrifice, seminal

plasma was collected and analyzed for AST, ALT, AP, AcP, and glutathione S-transferase (GST) activities. Histopathological evaluations of tissues were not conducted.

No clinical signs of toxicity were observed throughout the study. Mean body weight over the 10-week treatment period was significantly ($p < 0.05$) decreased by 9% compared to controls, although average food intake over the 10-week period was not affected by treatment; final body weight was not reported. After treatment for 10 weeks, relative testes and epididymis weights were significantly decreased by 22% ($p < 0.05$). The 10-week mean plasma testosterone level in treated rabbits was decreased by 21% ($p < 0.05$) compared with controls. In treated rabbits compared with controls, mean values of the following sperm-related characteristics were significantly ($p < 0.05$) decreased after 10 weeks: (1) packed sperm volume (10% decrease), (2) sperm concentration (18% decrease), (3) total sperm output (26% decrease), (4) sperm motility (5% decrease), (5) total motile sperm per ejaculation (34% decrease), (6) total functional sperm fraction (37% decrease), and (7) normal sperm (4% decrease). Both percentage of dead sperm (24% increase) and seminal fluid pH (4% increase) were increased; no effect was observed on semen ejaculate volume. Seminal fluid activities of GST, AST, and AcP were significantly ($p < 0.05$) decreased at the end of the treatment period, although decreases were small (≤12%) compared with controls.

The results indicate that exposure of rabbits to oral potassium dichromate gavage doses of 3.6 mg hexavalent chromium/kg-day for 10 weeks produced adverse effects on male reproductive tissues, including decreased testes and epididymis weight and decreased sperm output. Thus, EPA identified a LOAEL for hexavalent chromium of 3.6 mg/kg-day from this study.

NTP, 1996a,b

The NTP conducted studies to investigate the potential effects of dietary hexavalent chromium as potassium dichromate on male reproductive organs in Sprague-Dawley rats (NTP, 1996b) and BALB/c mice (NTP, 1996a). The NTP studies were designed to expand or replicate the Zahid et al. (1990) study (described above), and thereby provide data to either refute or confirm findings of adverse male reproductive effects from hexavalent chromium exposure.

Groups of 24 male and 48 female Sprague-Dawley rats were exposed to diets containing 0, 15, 50, 100, or 400 mg potassium dichromate/kg diet (equivalent to 0, 5.3, 17.6, 35.3, or 141.2 mg hexavalent chromium/kg diet, respectively) daily for 9 weeks followed by an 8-week recovery period (NTP, 1996b). Based on food consumption measured during the 9-week treatment period, NTP (1996a, b) calculated average daily doses of 0, 1, 3, 6, or 24 mg potassium dichromate/kg-day (equivalent to 0, 0.35, 1.1, 2.1, or 8.5 mg hexavalent chromium/kg-day) in males and 0, 1, 3, 7, or 28 mg hexavalent chromium/kg-day (equivalent to 0, 0.35, 1.1, 2.5, or 9.9 mg hexavalent chromium/kg-day) in females for the 0, 15, 50, 100, or 400 mg potassium dichromate/kg diet groups, respectively. Animals were examined twice daily for mortality and

clinical signs of toxicity. Physical examinations and measurement of body weight and food and water consumption were conducted weekly. After 3, 6, or 9 weeks of treatment or after the full recovery period, 6 males and 12 females were sacrificed; necropsies were performed; blood was obtained for hematology (i.e., Hgb, Hct, MCV, MCH, MCHC, mean platelet volume, and erythrocyte, leukocyte, and platelet counts); organ weights (not specified, but including right and left testes) were recorded; microscopic examinations were conducted on liver, kidney, ovary, and testes (testes and epididymis were examined for Sertoli nuclei and preleptotene spermatocyte counts in Stage X or XI tubules); and sperm were collected and analyzed for chromatin structure.

No mortalities or treatment-related clinical signs of toxicity were observed in rats in any treatment group (NTP, 1996b). Body weights and food and drinking water consumption were comparable between controls and treatment groups. Results of hematological analyses showed a slight erythrocyte microcytosis in the highest dose group, as indicated by small, but significant, decreases in MCV in females exposed for 3 weeks (3% decrease; $p < 0.05$) and in males exposed for 9 weeks (6% decrease; $p < 0.05$), compared with controls; at 9 weeks, MCV in females was decreased by 3%, but the change was not statistically significant. No changes in MCV were observed in rats exposed for 6 weeks or at the end of the 8-week recovery period. After 9 weeks of treatment, MCH was decreased by approximately 6% in males and females (statistical significance not reported). No treatment-related findings were observed on necropsy or on microscopic examination of the liver, kidney, ovary, testes, epididymis, or sperm. In conclusion, no adverse effects on reproductive organs were observed in male or female rats exposed to dietary potassium dichromate at doses of 8.5 and 9.9 mg hexavalent chromium/kg-day, respectively, for up to 9 weeks. Based on slight erythrocyte microcytosis, the study authors identified respective NOAELs and LOAELs of 2.1 and 8.5 mg hexavalent chromium/kg-day in male Sprague-Dawley rats, and 2.5 and 9.9 mg hexavalent chromium/kg-day in females.

Groups of 24 male and 48 female BALB/c mice were exposed to diets containing 0, 15, 50, 100, or 400 mg potassium dichromate/kg diet (equivalent to 0, 5.3, 17.6, 35.3, or 141.2 mg hexavalent chromium/kg diet, respectively) daily for 9 weeks followed by an 8-week recovery period (NTP, 1996a). Based on food consumption measured during the 9-week treatment period, the study authors calculated average daily doses of 0, 3, 10, 21, or 92 mg potassium dichromate/kg-day (equivalent to 0, 1.1, 3.5, 7.4, or 32.5 mg hexavalent chromium/kg-day) in males and 0, 5, 16, 34, or 137 mg hexavalent chromium/kg-day (equivalent to 0, 1.8, 5.6, 12.0, or 48.4 mg hexavalent chromium/kg-day) in females for the 0, 15, 50, 100, or 400 mg potassium dichromate/kg diet groups, respectively. This study followed the same protocol and conducted the same evaluations as described in the NTP (1996b) study in rats (described above).

Mortalities occurred in five male mice, but they were deemed not related to treatment, and no treatment-related findings were observed on necropsy. The number of deaths were one, one, two, one, and none in the control through high-dose male groups, respectively. All females survived to study completion. No treatment-related clinical signs of toxicity were observed. At

most weekly evaluations, body weight was decreased by 5–9% in males in the highest dose group and by 2–4% in females in the two highest dose groups (statistical significance not reported); body weights in these groups remained depressed during the post-treatment recovery period in high-dose males and in females at 12 mg hexavalent chromium/kg-day (but not high-dose females). Feed consumption was generally increased (5–34%, relative to controls) in all treatment groups in males, although changes were not statistically significant; in females, feed consumption was increased in all dose groups (1–37%), with changes of statistical significance in most dose groups during treatment weeks 5 and 6. Water consumption in males and females was decreased through the first 3 weeks of treatment and comparable to controls for the remainder of the exposure period. Hematological analyses showed a slight erythrocyte microcytosis. In high-dose male and female mice, MCV was decreased by 2–4% ($p < 0.05$) at weeks 3, 6, and 9; MCV was also slightly decreased (<2%) at 12 mg hexavalent chromium/kg-day in females at 6 weeks. Changes in MCV were generally accompanied by small decreases in MCH. At the end of the recovery period, a small increase in MCV (2.8%; $p < 0.05$) was observed in males; in females, MCV in all treatment groups was comparable to controls. No other effects on hematological parameters were observed. Microscopic evaluations revealed a treatment-related increase in the incidence of cytoplasmic vacuolization of hepatocytes in male and female mice at the end of the 9-week treatment period. Vacuoles were demarked and appeared small and clear; NTP (1996a) noted that vacuoles were consistent with lipid accumulation. Incidences of hepatic cytoplasmic vacuolization in the control through high-dose groups were 0/6, 0/6, 1/6, 2/6, and 2/5 in males and 1/12, 0/12, 3/12, 2/12, and 4/12 in females, respectively; lesion severity and statistical significance were not reported. No other treatment-related histopathological findings were observed.

In conclusion, no adverse effects on reproductive organs were observed in male or female mice exposed to dietary potassium dichromate at doses up to 32.5 and 48.4 mg hexavalent chromium/kg-day, respectively, for 9 weeks. Based on histopathological changes to the liver (cytoplasmic vacuolization), the study authors identified a NOAEL of 1.1 mg hexavalent chromium/kg-day for males and 1.8 mg hexavalent chromium/kg-day for females.

4.3.2. Effects on Reproductive Outcomes

Elbetieha and Al-Hamood, 1997

Reproductive effects of drinking water containing 1,000–5,000 mg potassium dichromate/L (equivalent to 353–1,765 mg hexavalent chromium/L) were evaluated in Swiss mice in a series of three experiments (Elbetieha and Al-Hamood, 1997). No data on drinking water consumption were included in the study report. Based on findings of other studies (NTP, 2008, 2007) showing decreased drinking water consumption and body weight at drinking water concentrations ≥30 mg hexavalent chromium/L, it is likely that drinking water consumption was decreased in all chromium treatment groups; thus, daily doses of hexavalent chromium cannot be

accurately estimated for this study. In the first experiment, sexually mature (i.e., 50 days old) male Swiss mice were exposed to drinking water containing 0 (20 males), 1,000 (19 males), 2,000 (11 males), 4,000 (9 males), or 5,000 (13 males) mg potassium dichromate/L (equivalent to 0, 353, 706, 1,412, or 1,765 mg hexavalent chromium/L, respectively) for 12 weeks. After 12 weeks, males were mated with untreated sexually mature females for 10 days; 1 week after completion of the mating period, females were sacrificed and evaluated for the number of pregnant females, viable fetuses, resorptions, and dead fetuses. Histopathological evaluations of tissues were not conducted. No data on body weights were reported. Exposure of male mice to hexavalent chromium did not affect the percentage of pregnant females. The numbers of implantations and viable fetuses were significantly reduced from 33% in controls to 20% ($p < 0.01$) and 16% ($p < 0.05$) in the 706 and 1,412 mg hexavalent chromium/L groups, respectively; in the 1,765 mg hexavalent chromium/L group, the numbers of implantation and viable fetuses were reduced to 19%, although this reduction did not reach statistical significance. No resorptions or dead fetuses were observed in the control, 706, or 1,412 mg potassium dichromate/L groups, but three resorptions were observed at 353 mg hexavalent chromium/L and six resorptions and six dead fetuses were observed at 1,765 mg hexavalent chromium/L (statistical significance not reported).

In the second experiment, sexually mature (i.e., 50 days old) female Swiss mice were exposed to drinking water containing 0 (19 females), 2,000 (15 females), or 5,000 (11 females) mg potassium dichromate/L (equivalent to 0, 706, or 1,765 mg hexavalent chromium/L, respectively) for 12 weeks (Elbetieha and Al-Hamood, 1997). After 12 weeks, each female was mated with an untreated sexually mature male for 10 days; 1 week after completion of the mating period, females were sacrificed and evaluated for the numbers of pregnant females, viable fetuses, and resorptions and dead fetuses. No data on body weights were reported. No treatment-related effects were observed on the number of pregnant mice. The number of implantations was significantly reduced from 17% in controls to 14% ($p < 0.01$) and 9% ($p < 0.05$) in the 706 and 1,765 mg hexavalent chromium/L groups, respectively, and the number of viable fetuses was significantly reduced from 17% in controls to 9% in the 706 ($p < 0.05$) and 1,765 ($p < 0.01$) mg hexavalent chromium/L groups, respectively. The number of mice with resorptions was significantly increased from 11% in controls to 53% ($p < 0.01$) and 63% ($p < 0.005$) in the 706 and 1,765 mg hexavalent chromium/L groups, respectively, and the total number of resorptions was increased from 4 in controls to 36 and 14 in the 706 and 1,765 mg hexavalent chromium/L groups, respectively (statistical significance not reported).

In the third experiment, sexually mature (i.e., 50 days old) mice were exposed to drinking water containing 0 (10 males, 8 females), 2,000 (13 males, no females), or 5,000 (13 males, 10 females) mg potassium dichromate/L for 12 weeks (Elbetieha and Al-Hamood, 1997). Following treatment, body weights and weights of reproductive organs (paired testes, seminal vesicles, preputial glands, paired ovaries, and uteri) were determined. No mortalities or clinical

signs of toxicity were observed. Final body weights of males were significantly ($p < 0.01$) reduced by approximately 10 and 12% in the 706 and 1,765 mg hexavalent chromium/L groups, respectively; final mean body weights of treated females were similar to controls. Relative testes weights were increased by approximately 18% ($p < 0.01$) and 22% ($p < 0.05$) in the 706 and 1,765 mg hexavalent chromium/L groups, respectively, and relative weights of seminal vesicles and preputial gland were significantly ($p < 0.001$) decreased by approximately 27 and 34%, respectively, in the 1,765 mg hexavalent chromium/L group. Relative ovary weight was significantly increased by 54% in females in the 1,765 mg hexavalent chromium/L group, although uterine weight was unaffected by treatment. Histopathological assessments of reproductive tissues were not conducted.

In conclusion, results of the three experiments conducted by Elbetieha and Al-Hamood (1997) show that exposure to potassium dichromate in drinking water affects reproductive outcomes in exposed males and females. In female mice, decreased numbers of implantations and viable fetuses and increased resorptions were observed at 2,000 mg potassium dichromate/L (equivalent to 706 mg hexavalent chromium/L). In males, exposure for 12 weeks prior to mating reduced the numbers of implantations and viable fetuses at 2,000 and 4,000 mg potassium dichromate/L (equivalent to 706 and 1,412 mg hexavalent chromium/L, respectively), but not at 1,000 mg potassium dichromate/L (equivalent to 353 mg hexavalent chromium/L). In addition, treatment-related changes in weights of male reproductive organs were observed at 2,000 and 5,000 mg potassium dichromate/L (equivalent to 706 and 1,412 mg hexavalent chromium/L, respectively). Although reproductive performance was not affected at the lowest exposure level, weights of male reproductive organs were not evaluated in male mice treated with 1,000 mg potassium dichromate/L. Due to inadequate reporting (i.e., no information on effects of treatment on body weight or drinking water consumption), EPA could not identify NOAEL or LOAEL values from this study.

NTP, 1997

The potential reproductive toxicity of dietary potassium dichromate was evaluated in BALB/c mice in a continuous breeding study (NTP, 1997). Groups of 20 male and female pairs (F_0) were exposed to dietary potassium dichromate at 0, 100, 200, and 400 mg potassium dichromate/kg diet (equivalent to 0, 17.6, 35.3, or 141.2 mg hexavalent chromium/kg diet, respectively) for 13 weeks (1 week prior to and 12 weeks during cohabitation). During exposure of the F_0 generation, animals were examined daily for mortality and clinical signs of toxicity; body weights and food consumption were measured periodically (4–5 times). Litters produced during the cohabitation period were evaluated (i.e., total pups, live and dead pups, and sex), weighed on postnatal day (PND) 1, and euthanized with no additional assessments; pregnancy index (number of litters/breeding pair) was also determined. After the cohabitation period, F_0 breeding pairs were separated and continued on study diets; litters born during the post-

separation period (F_1 animals) were reared with the F_0 dams until weaning (PND 21). Dam and pup weights and dam food consumption were monitored during the lactational period. Upon weaning, F_0 animals were sacrificed and the following terminal evaluations were conducted: necropsy; organ weights (liver, kidneys, right cauda epididymis, right epididymis, prostate, seminal vesicles with coagulating glands, right testis, and ovaries); sperm evaluations (testicular spermatid head count and epididymal sperm density, motility, and morphology); and histopathology (liver and kidneys). Following weaning of F_1 animals, animals were maintained on the same study diets as their parents. During postlactational exposure of the F_1 generation, animals were examined daily for mortality and clinical signs of toxicity; body weights and food consumption were measured periodically (3–4 times). At sexual maturity (approximately 74 days), groups of 20 F_1 animals of each sex were selected as breeding pairs (avoiding sibling matings), cohabitated for 7 days, and then separated. Reproductive endpoints (numbers of live and dead pups, sexes of pups, and total pup weight by sex) were evaluated on PND 1 of the F_2 offspring; there was no further evaluation of the F_2 pups. Estrous cycle (time spent in estrous stages, cycle length, number of cycles, number of cycling females, and number of females with regular cycles) was evaluated using 12-day vaginal smears beginning 4 days after the last delivery. Terminal evaluations of F_1 adults (time from separation to terminal sacrifice not reported) were the same as those described above for F_0 adults, with the addition of hematology (i.e., Hgb, Hct, MCV, MCH, MCHC, mean platelet volume, erythrocyte morphology, and erythrocyte, leukocyte, and platelet counts).

No treatment-related mortalities or clinical signs of toxicity were observed in F_0 generation BALB/c mice exposed to dietary potassium dichromate (NTP, 1997). Mortalities occurred in eight animals (four low-dose males, one mid-dose male, and three mid-dose females); however, since no mortalities were observed in the high-dose group, NTP (1997) concluded that these deaths were not related to treatment. Terminal body weight of males in all treatment groups was comparable to controls; mean body weight of females in the high-dose groups was decreased by 7% ($p < 0.05$). In general, food consumption was increased in treatment groups. Based on measured food consumption and body weights during the cohabitation period, NTP (1997) calculated average daily doses in F_0 males and females of 0, 19.4, 38.6, or 85.7 mg potassium dichromate/kg-day (equivalent to 0, 6.8, 13.6, or 30.3 mg hexavalent chromium/kg-day, respectively). During lactation, sporadic decreases in body weights of dams in the mid- and high-dose groups were observed, but body weights at the end of lactation (PND 21) were similar to controls; food consumption during lactation was similar between control and treatment groups. Based on measured food consumption and body weights, NTP (1997) calculated average daily doses in lactating F_0 females of 0, 32.8, 69.0 or 143.1 mg potassium dichromate/kg-day (equivalent to 0, 11.6, 24.4, or 50.5 mg hexavalent chromium/kg-day, respectively). At the terminal evaluations of F_0 animals, absolute (but not relative) liver weights were increased by 17% ($p < 0.05$) and 22% ($p < 0.05$) in high-dose males and females,

respectively, compared with controls. No other changes in organ weights were observed. No treatment-related histopathological findings were observed in the F_0 generation. Although various hepatic lesions were observed, including cytoplasmic vacuolization, the study authors concluded that these findings were not treatment-related, since incidence data did not show a relationship with dose. Evaluations of male reproductive tissues did not reveal any treatment-related effects. In the F_0 generation, no treatment-related effects on reproductive outcomes, including pregnancy index, mean cumulative time to litter, litter size, live and dead pups/litter, live pup weight, and sex ratio, were observed.

Evaluations conducted on F_1 pups during lactational exposure showed no effects on pup survival (NTP, 1997). On PND 21, weight of high-dose male pups was decreased by 16% compared with controls, but the decrease was not statistically significant. From weaning to sexual maturity, two mortalities occurred (one control male and one high-dose male). No treatment-related clinical signs of toxicity were observed. At the initiation of the F_1 breeding phase (approximately PND 74), mean body weights of mid-dose females were decreased by 6% compared with controls and by 9% in high-dose F_1 males and females (statistical significance not reported). Food consumption was generally increased during the period from weaning to sexual maturity. Based on measured food consumption and body weights, NTP (1997) calculated average daily doses in F_1 animals of 0, 22.4, 45.5 or 104.9 mg potassium dichromate/kg-day (equivalent to 0, 7.9, 16.1, or 37.1 mg hexavalent chromium/kg-day, respectively). Hematological analysis at terminal sacrifice of F_1 adults revealed slight erythrocyte microcytosis based on the following observations (comparisons to controls, statistical significance not reported): MCV decreased by 3% in mid- and high-dose males and by 2, 3, and 4% in low-, mid-, and high-dose females, respectively; MCH decreased by 3% in high-dose males; and Hgb decreased by 5% in high-dose F1 females. No changes in erythrocyte morphology were observed. Relative kidney weight was increased by 5% in mid-dose females, but no other organ weight changes were observed. No treatment-related histopathological findings were observed. Although various hepatic lesions were observed, including cytoplasmic vacuolization, NTP (1997) concluded that findings were not treatment-related, since incidence data did not show a relationship with dose. Evaluations of male reproductive tissues and female estrous cycle did not reveal any treatment-related effects. In the F_1 generation, no treatment-related effects on reproductive outcomes, including pregnancy index, mean cumulative time to litter, gestation length, litter size, live and dead pups/litter, and sex ratio, were observed. Live pup weight of females in the high-dose group was decreased by 11% ($p < 0.05$) compared to controls, but no decrease was observed for live pup weight of males or of combined males and females.

In conclusion, NTP (1997) identified a LOAEL for parental toxicity in the F_1 generation of 7.9 mg hexavalent chromium/kg-day in females exposed to potassium dichromate in the diet based on erythrocyte microcytosis (slight decrease in MCH); a NOAEL for parental toxicity in the F_1 generation was not established because effects were seen at the lowest dose tested.

Although NTP (1997) did not specifically identify a NOAEL for reproductive effects, in the absence of reproductive findings, the highest dose tested in this study is identified by EPA as a free-standing NOAEL for effects of dietary hexavalent chromium exposure on fertility and on male and female reproductive organ histology and weights (i.e., 30.3 mg hexavalent chromium/kg-day in F_0 mice and 37.1 mg hexavalent chromium/kg-day in F_1 mice).

4.3.3. Effects of Pregestational Exposure on Reproductive Outcome and Fetal Development

Kanojia et al., 1996

Kanojia et al. (1996) administered adult Swiss albino female rats (20/group) drinking water containing 0, 250, 500, or 750 mg hexavalent chromium/L (as potassium dichromate) for 20 days prior to gestation. During the exposure and gestational periods, body weights and water intake were recorded daily. At the end of the exposure period, rats were mated overnight with untreated males. Following mating, the mating index (percentage of mated females) and the fertility index (percentage of pregnant females) were determined. On GD 19, 10 rats/group were sacrificed and the numbers of copora lutea, fetuses/litter, live and dead fetuses, and resorptions; pre- and postimplantation losses; and fetal and placental weights were recorded and fetuses were examined for internal abnormalities (one third of fetuses) and external and skeletal abnormalities (remaining fetuses). In the remaining 10 rats/group, estrous cycle length was evaluated for 12 consecutive cycles. Based on drinking water consumption during the exposure period, Konijia et al. (1996) reported daily hexavalent chromium intakes of 6.4, 12.2, and 15.3 mg hexavalent chromium/rat-day. The study report did not include data on body weights over the course of the 20-day treatment period, although it is likely that treatment-related effects on body weight occurred during the exposure period, as significant decreases in gestational weight gain were observed in all treatment groups (decreases of approximately 8, 14, and 21% in the low-, mid-, and high-dose groups, respectively, compared to controls). Thus, in the absence of data on the effect of treatment on body weights during the exposure period, daily doses of hexavalent chromium in terms of body weight (e.g., mg hexavalent chromium/kg-day) cannot be accurately estimated.

No mortalities or clinical signs of toxicity in dams were observed. Dose-related decreases in mating and fertility indices were observed; in the high-dose group, mating and fertility indices were decreased by 60 and 68%, respectively, compared to controls (statistical significance not reported). In all treatment groups, the number of live fetuses was decreased, the numbers of resorptions and postimplantation loss were increased, and placental weight was increased. In the mid- and high-dose groups, numbers of corpora lutea and implantations were decreased and preimplantation losses were increased. No treatment-related effects were observed for fetal weight or crown-rump length. Examination of fetuses showed gross abnormalities in the high-dose group, including patches of subdermal hemorrhage, kinky tail,

short tail, and dropping wrist. Skeletal abnormalities were also observed, including reduced caudal ossification in mid- and high-dose groups and reduced parietal and inter-parietal ossification in the high-dose group. No visceral abnormalities were observed. Postpartum estrous cycle length was significantly increased by 37% ($p < 0.05$) in the high-dose group.

Results of this study show that 20-day pregestational exposure of Swiss albino rat dams to hexavalent chromium adversely affected reproductive outcomes (decreased number of live fetuses and increased number of resorptions and postimplantation loss) at the lowest drinking water concentrations of potassium dichromate tested (≥250 mg hexavalent chromium/L or ≥6.4 mg hexavalent chromium/rat-day) and produced adverse developmental effects (gross and skeletal abnormalities) at the highest drinking water concentrations tested (750 mg hexavalent chromium/L or 15.3 mg hexavalent chromium/rat-day). Because of the lack of reporting of body weight data over the course of the study, NOAELs and/or LOAELs, expressed in mg hexavalent chromium/kg-day, could not be derived from this study by EPA.

Kanojia et al., 1998

Kanojia et al. (1998) administered adult Druckrey female rats (20/group; mean initial body weight 80 g) drinking water containing 0, 250, 500, or 750 mg hexavalent chromium/L (as potassium dichromate) for 3 months prior to gestation. This study was designed to follow the same protocol as that used in the Kanojia et al. (1996) study (described above). However, at the end of the 3-month exposure period, rats in all treatment groups were acyclic (persistent diestrous phase). Therefore, since mating could not take place immediately following completion of the exposure period, rats were held for an additional 15–20 days (treatment-free), during which time the estrous cycle resumed.

During the exposure period, mortality occurred in 15 and 10% of rats in the mid- and high-dose groups, respectively; no deaths occurred in the control or low-dose groups. Clinical signs of toxicity observed during the exposure period in the mid- and high-dose groups included hair loss and lethargic and aggressive behavior. At the end of the exposure period, body weight was significantly ($p < 0.05$) decreased by approximately 18 and 24% in the mid- and high-dose groups, respectively, compared with controls. Kanojia et al. (1998) reported average hexavalent chromium intakes (based on water consumption) of 5.57, 10.18, and 13.56 mg hexavalent chromium/rat-day in the low-, mid-, and high-dose groups, respectively. Using these daily intake levels and the mean initial body weight of 80 g, daily doses of 70, 127, and 170 mg hexavalent chromium/kg-day for the low-, mid-, and high-dose groups, respectively, were estimated. During the post-exposure gestational period, maternal weight gain was significantly ($p < 0.05$) decreased by 17 and 22% in the mid- and high-dose groups, respectively, compared with controls. The mating index was decreased by 30, 40, and 60% and the fertility index was decreased by 32, 41, and 49% in the low-, mid-, and high-dose groups, respectively, compared with controls (statistical significance not reported). In all treatment groups, pre- and

postimplantation losses were significantly ($p < 0.05$) increased, with increases in the high-dose group reaching 3.1- and 4.2-fold, respectively. In the mid- and high-dose groups, the numbers of implantations, live fetuses, and resorptions were significantly ($p < 0.05$) increased. Assessments of fetuses (on a per litter basis compared with controls) showed the following (significant difference compared with controls; $p < 0.05$): decreased fetal weight (all treatment groups); decreased crown-rump length (mid- and high-dose groups); gross external abnormalities, including subdermal hemorrhagic patches and drooping wrists in all treatment groups and kinky and short tail in mid- and high-dose groups; and skeletal abnormalities, including decreased caudal ossification in all treatment groups and reduced parietal and interparietal ossification in mid- and high-dose groups. No internal abnormalities in fetuses were observed. Postpartum estrous cycle length was significantly ($p < 0.05$) increased in all treatment groups, with increases reaching approximately 1.7-fold in the high-dose group.

Results of this study show that 3-month pregestational exposure of Druckrey rat dams to hexavalent chromium as potassium dichromate adversely affected reproductive outcomes (increased pre- and postimplantation losses) and produced adverse developmental effects (decreased fetal weight and external and skeletal abnormalities) at all drinking water concentrations tested (≥250 mg hexavalent chromium/L or approximately ≥70 mg hexavalent chromium/kg-day). Thus, a LOAEL of 70 mg hexavalent chromium/kg-day was identified from this study by EPA.

Junaid et al., 1996a

Junaid et al. (1996a) administered Swiss albino female mice drinking water containing 0, 250, 500, or 750 mg hexavalent chromium/L (as potassium dichromate) from days 6 to 14 of gestation. The study followed the same protocol and conducted the same evaluations as those reported in the study by Kanojia et al. (1996) (described above), except that estrous cycle length was not evaluated. Evaluations on reproductive outcomes and developmental effects were conducted in 10 mice/group.

No clinical signs of toxicity were observed in mice during the exposure period. In the high-dose group, mortality occurred in 20% of animals; the cause of death was not established. Based on drinking water consumption monitored during the exposure period, the study authors reported daily hexavalent chromium intake levels of 1.9, 3.56, and 5.23 mg hexavalent chromium/mouse-day in the low-, mid-, and high-dose groups, respectively. No treatment-related effects were observed on body weight (data not reported); thus, using the reported mean initial body weight of 30 g, daily doses of 63, 119, and 174 mg hexavalent chromium/kg-day for the low-, mid-, and high-dose groups, respectively, were estimated. During the gestational period, maternal weight gain in the low- and mid-dose groups was comparable to controls; no weight gain was observed during gestation in high-dose group dams. In the low-dose group, postimplantation loss was significantly ($p < 0.05$) increased compared with controls (control:

0%; low-dose group: 17.5%); no effects were observed for the numbers of corpora lutea, implantations, live fetuses, or resorptions or for preimplantation loss. In the mid-dose group, the numbers of implantation and live fetuses were significantly ($p < 0.05$) decreased and the numbers of resorptions and pre- and postimplantation losses were significantly ($p < 0.05$) increased; no effect on the number of corpora lutea was observed. In the high-dose group, no litters were produced and implantation sites were completely absent; corpora lutea were present, but numbers were decreased by 44% compared to controls. Assessments of fetuses (on a per litter basis compared with controls) showed the following (significant difference compared to controls; $p < 0.05$): decreased fetal weight and length in the low- and mid-dose groups; gross (external) abnormalities, including subdermal hemorrhagic patches and short and kinky tail in the mid-dose group; and skeletal abnormalities, including reduced caudal ossification in the low- and mid-dose groups and reduced parietal and interparietal ossification in the mid-dose group. No internal abnormalities in fetuses were observed.

Thus, at all drinking water concentrations of potassium dichromate tested (≥250 mg hexavalent chromium/L or approximately ≥63 mg hexavalent chromium/kg-day), pregestational exposure of Swiss albino female mice for 20 days produced adverse effects on reproductive outcome (decreased fertility) and fetal development (decreased fetal body weight and delays in skeletal development). Thus, EPA identified a LOAEL of 63 mg hexavalent chromium/kg-day from this study.

4.3.4. Effects of Gestational and/or Lactational Exposure on Reproductive Outcome and Fetal Development

Elsaieed and Nada, 2002

Effects of gestational exposure to hexavalent chromium were investigated in Wistar rats (Elsaieed and Nada, 2002). Groups of 10 pregnant rats (mean initial body weight of 170 g) were administered drinking water containing 0 or 50 mg hexavalent chromium/L as potassium dichromate on GDs 6 through 15. During the exposure period, dams were evaluated for clinical signs of toxicity, body weights, and food and drinking water consumption. One day before delivery, rats were sacrificed and the following were evaluated: numbers of corpora lutea, pre- and postimplantation losses, resorptions, and live and dead fetuses; fetal weight; and visceral and skeletal anomalies.

No mortalities or clinical signs of toxicity were observed. Elsaieed and Nada (2002) stated that food and drinking water consumption was comparable between control and treatment groups, although data were not reported. Gestational weight gain was significantly ($p < 0.05$) decreased by 40% in treated dams, compared with controls. Based on an average gestational body weight of 177 g (average calculated using body weights at mating and at the end of gestation) and the allometric equation for drinking water consumption for laboratory mammals ($0.10 \times \text{body weight}^{0.7377}$; U.S. EPA, 1988), a daily dose of 7.9 mg hexavalent chromium/kg-day

was estimated by EPA. In this study, treatment of rats with hexavalent chromium resulted in significant ($p < 0.05$) increases in preimplantation loss/litter (2.1 vs. 0 in control), postimplantation loss/litter (1.5 vs. 0), resorptions/litter (1.2 vs. 0), and dead fetuses/litter (1.2 vs. 0) and decreases in live fetuses/litter (1.5 vs. 6.8 in control) and fetal weight (33% decrease). In the exposed group, increased litters with fetal abnormalities or malformations were observed including visceral (renal pelvis dilation: 2.1/litter) and skeletal (incomplete skull ossification: 1.0/litter) changes; no control fetuses showed these changes.

The results showed that exposure of pregnant Sprague-Dawley rats to drinking water containing 50 mg hexavalent chromium/L as potassium dichromate (approximately 7.9 mg hexavalent chromium/kg-day) on GDs 6–15 produced adverse effects on reproductive outcome and fetal development. Thus, EPA identified a LOAEL of 7.9 mg hexavalent chromium/kg-day from this study.

Bataineh et al., 2007

Reproductive outcomes were evaluated in adult female rats (age not specified) orally exposed to potassium dichromate for 3 days following mating (Bataineh et al., 2007). Groups of 10 successfully mated female Sprague-Dawley rats were administered daily doses of 0 or 25 mg potassium dichromate/rat (equivalent to 8.8 mg hexavalent chromium/rat-day or approximately 35 mg hexavalent chromium/kg-day, based on the average reported body weight of 245 g at mating) in saline daily by gavage on GDs 1–3 or 4–6. On GD 20, rats were sacrificed and the numbers of implantation sites, live fetuses, and resorptions along the uterine horns were recorded; fetuses were not assessed for external, skeletal, or visceral abnormalities.

In rats treated with potassium dichromate on GDs 1–3, no pregnancies, implantations, resorptions, or viable fetuses were observed, compared with 10/10 pregnancies, 8.2 implantations/female, 8.2 live fetuses/female, and 0/82 resorptions in controls. In rats treated on GDs 4–6, the numbers of pregnant rats and implantations/female were comparable to values in the control group. However, the number of viable fetuses was decreased by 69% ($p < 0.001$) and the percentage of resorptions per implantations was increased by 222% ($p < 0.001$). The study report did not indicate if clinical signs of toxicity were observed in chromium-treated dams, and no additional measures to assess systemic toxicity were reported.

The results indicate that short-term gavage exposure of Sprague-Dawley rat dams to potassium dichromate at a dose of 35 mg hexavalent chromium/kg-day on GDs 1–3 completely impaired implantation; exposure on GDs 4–6 markedly increased resorptions and decreased the number of viable fetuses, compared with controls. Thus, a LOAEL of 35 mg hexavalent chromium/kg-day was identified from this study by EPA.

Trivedi et al., 1989

Effects on reproductive outcome and fetal development were observed in ITRC-bred albino mice administered hexavalent chromium in drinking water (Trivedi et al., 1989). Groups of 10–13 pregnant mice (average initial body weight of 30 g) were administered drinking water containing 0, 250, 500, or 1,000 mg hexavalent chromium/L (as potassium dichromate) during the entire gestational period. Dams were observed daily for mortality, clinical signs of toxicity, body weight, and water consumption. On GD 19, dams were sacrificed and the following were recorded: numbers of corpora lutea, total implantations, live and dead fetuses, and pre-implantation and postimplantations losses; placental weight; fetal weight and crown-rump length; number of stunted fetuses; and sex ratio per litter. In addition, fetuses were examined for external (all fetuses), internal (approximately one-third of fetuses), and skeletal (remaining fetuses) anomalies.

No mortalities or clinical signs of toxicity were observed in the dams. In the low-dose group, body weight gain was comparable to controls; however, body weight gain was significantly decreased by 21% ($p < 0.05$) in the mid-dose group, and dams in the high-dose group lost weight during treatment. Daily hexavalent chromium intakes were reported as 1.76, 3.6, and 7.03 mg hexavalent chromium/mouse-day in the low-, mid-, and high-dose groups, respectively, based on measured drinking water consumption. Using average body weights for the gestational period (36.8, 36.6, and 29.4 g in the low-, mid-, and high-dose groups, respectively; calculated for this review using: [average initial body weight + body weight at the end of gestation]/2) and reported daily chromium intakes, daily doses of 48, 98, and 239 mg hexavalent chromium/kg-day were estimated. In low-dose mice, the percentages of resorptions and postimplantation loss were significantly increased ($p < 0.001$) to 33 and 36%, respectively, compared with 10 and 1.7%, respectively, in controls; the number of litters, litter size, number of copora lutea, and placental weight in the low-dose group were comparable to controls. In the mid-dose group, the percentages of resorptions and postimplantation losses were significantly ($p < 0.001$) increased to 52 and 88%, respectively. In addition, in the mid-dose group, litter size was significantly decreased by 44% ($p < 0.01$) compared with controls, and the percentage of preimplantation loss was increased to 26.2% ($p < 0.001$), compared with 3.6% in controls. No treatment-related effects on placental weight were observed in the low- or mid-dose groups. In the high-dose group, no litters were produced and implantation sites were completely absent. In the low- and mid-dose groups, mean fetal crown-rump lengths were decreased ($p < 0.001$) by 17 and 27%, respectively, and mean fetal weights were decreased ($p < 0.001$) by 31 and 44%, respectively. Sex ratio was unaffected by treatment. Examination of fetuses for external anomalies showed no effects in the low-dose group; in the mid-dose group, tail kinking and subdermal hemorrhagic patches and streaks were observed. An increase in the incidence of minor skeletal anomalies was observed in fetuses in the low-dose (reduced ossification of the cranium) and mid-dose (reduced ossification of the cranium, forelimb, hindlimb, sternebrae, and

thoracic and caudal vertebrae and reduced number of ribs) groups. No internal anomalies were observed.

From this study, EPA identified a LOAEL and NOAEL for maternal toxicity, and a LOAEL for developmental effects. The LOAEL and NOAEL values for maternal toxicity, based on decreased body weight gain in ITRC-bred albino mice exposed to potassium dichromate in drinking water throughout gestation, were 98 and 48 mg hexavalent chromium/kg-day, respectively. Based on increased resorptions and postimplantation loss, and decreased fetal length and weight, the lowest concentration tested (250 mg hexavalent chromium/L or 48 mg hexavalent chromium/kg-day) was identified as a LOAEL for developmental effects.

Junaid et al., 1996b

Junaid et al. (1996) evaluated the effects of oral exposure of pregnant mice to hexavalent chromium on reproductive outcome and fetal development. Groups of 10 successfully mated Swiss albino female mice (average initial body weight of 30 g) were administered drinking water containing 0, 250, 500, or 750 mg hexavalent chromium/L (as potassium dichromate) on GDs 6 though 14. Throughout the exposure period, dams were evaluated daily for clinical signs of toxicity, body weight, and drinking water consumption. On GD 19, dams were sacrificed and evaluations of dams and fetuses were conducted as described by Trivedi et al. (1989) (summarized above).

No mortalities or clinical signs of toxicity were observed in the dams. Gestational weight gain was significantly ($p < 0.05$) decreased in the mid- and high-dose groups by 8 and 32%, respectively, but was comparable to controls in the low-dose group. Daily hexavalent chromium intakes were reported as 2.00, 3.75, or 5.47 mg chromium/mouse-day in the low-, mid-, and high-dose groups, respectively, based on measured drinking water consumption. Using average body weights for the gestational period (37.6, 37.2, and 35.9 g in the low-, mid-, and high-dose groups, respectively; calculated for this report using: [average initial body weight + body weight at the end of gestation]/2) and reported daily chromium intakes, daily doses of 53, 101, and 152 mg hexavalent chromium/kg-day in the low-, mid-, and high-dose groups, respectively, were estimated. The number of resorptions was significantly ($p < 0.05$) increased in all treatment groups, with increases reaching 7.7-fold in the high-dose group. In the mid- and high-dose groups, significant ($p < 0.05$) decreases in the total number of fetuses and increases in the numbers of dead fetuses and resorption sites were observed. Fetal weight was significantly ($p < 0.05$) decreased by 13 and 19% in the mid- and high-dose groups, respectively; no treatment-related effects were observed on fetal length. Gross external examination of fetuses showed significant ($p < 0.05$) increases in the incidences of minor abnormalities (subdermal hemorrhagic patches, drooping wrist, kinky and short tail) in the high-dose group. Examination of fetuses for skeletal abnormalities showed significant ($p < 0.05$) increases in the incidences of reduced caudal ossification in the mid- and high-dose groups and of reduced nasal, frontal,

parietal, interparietal, carpals, and tarsals ossification. No external or skeletal abnormalities were observed in fetuses in the low-dose group. No visceral abnormalities were observed in any treatment group.

Junaid et al. (1996b) concluded that oral exposure of dams during the organogenesis phase of gestation produced adverse effects in embryos and during fetal development. EPA identified LOAEL and NOAEL values for maternal toxicity of 101 and 53 mg hexavalent chromium/kg-day, respectively, based on decreased body weight gain in Swiss albino mice administered potassium dichromate in drinking water on GDs 6–14. Based on reduced number of implantation sites, the lowest dose tested (approximately 53 mg hexavalent chromium/kg-day) in this study was identified by EPA as a developmental LOAEL.

Junaid et al., 1995

The effects of late gestational exposure to hexavalent chromium on reproductive outcome and fetal development were evaluated in mice (Junaid et al., 1995). Groups of 10 successfully mated Swiss albino female mice (average initial body weight of 30 g) were administered drinking water containing 0, 250, 500, or 750 mg hexavalent chromium/L (as potassium dichromate) on GDs 14 though 19. Throughout the exposure period, dams were evaluated daily for clinical signs of toxicity, body weight, and drinking water consumption. On GD 19, dams were sacrificed and evaluations of dams and fetuses were conducted as described by Trivedi et al. (1989) (summarized above).

No mortalities or clinical signs of toxicity were observed in the dams. Gestational weight gain was significantly ($p < 0.05$) decreased in the mid- and high-dose groups by 11 and 26%, respectively, but was comparable to controls in the low-dose group. No data on drinking water consumption were reported; however, it is likely that daily doses were similar to those calculated for the study by Junaid et al. (1996b) (e.g., approximately 53, 101, and 152 mg hexavalent chromium/kg-day in the low-, mid-, and high-dose groups, respectively), which used the same mouse strain and drinking water concentrations, and a similar study design. In the mid- and high-dose groups, the numbers of dead fetuses and postimplantation losses were significantly ($p < 0.05$) increased; the numbers of corpora lutea and total fetuses per litter were similar to controls in all treatment groups. Fetal weight and length were significantly decreased in all treatment groups, with decreases reaching approximately 47 and 29%, respectively, in the high-dose group. Gross external examination of fetuses showed significant ($p < 0.05$) increases in the incidences of minor abnormalities in the mid-dose (drooping wrists) and high-dose (drooping wrists, subdermal hemorrhagic patches, kinky and short tail) groups. Examination of fetuses for skeletal abnormalities showed significant ($p < 0.05$) increases in the incidences of reduced caudal ossification in all treatment groups, reduced tarsals ossification in mid- and high-dose groups, and reduced nasal, parietal, interparietal, carpals, and metatarsals ossifications in the high-dose group. No visceral abnormalities were observed in any treatment group.

From this study, EPA identified a LOAEL and NOAEL for maternal toxicity, and a LOAEL for developmental effects. The NOAEL and LOAEL values for maternal toxicity, based on decreased body weight gain in Swiss albino mice administered potassium dichromate in drinking water on GDs 14–19, were 53 and 101 mg hexavalent chromium/kg-day, respectively. Based on reduced fetal weight and length and increased incidence of reduced caudal ossification in all treatment groups, the lowest dose tested (approximately 53 mg hexavalent chromium/kg-day) was identified as a developmental LOAEL.

Al-Hamood et al., 1998

The effects of gestational and lactational exposure of mice to hexavalent chromium on sexual maturation and fertility in offspring were investigated by Al-Hamood et al. (1998). On GD 12 through day 20 of lactation, groups of 25 pregnant Swiss strain BALB/c mice (mean initial body weight of 25 g) were administered drinking water containing 0 or 1,000 mg potassium dichromate/L (equivalent to 353 mg hexavalent chromium/L). Based on drinking water consumption by dams, daily hexavalent chromium intakes of 2.1 and 1.7 mg hexavalent chromium/mouse-day were calculated for the gestational and lactational periods, respectively. No data on body weights of dams were reported; however, since other studies have shown decreased maternal weight gain in pregnant mice exposed to drinking water containing ≥176 hexavalent chromium/L) (Junaid et al., 1996b, 1995), it is likely that treatment-related decreases in maternal weight gain occurred. Therefore, given this uncertainty, daily hexavalent chromium doses expressed in terms of body weight cannot be accurately estimated for this study. At birth, litters were culled to eight pups per female and offspring were weaned on PND 21; from weaning to day 60 of age, offspring received control drinking water. From PND 20 to the onset of puberty, female offspring were examined for time to vaginal opening. Fertility in offspring was assessed at day 60 of age; male offspring were mated with untreated females and female offspring were mated with untreated males for 10 days. At completion of the mating period, females were examined for numbers of pregnant females, implantations, viable fetuses, and resorptions. Additional groups (n = 9–12) of offspring were sacrificed on day 50 of age, and body weights and weights of reproductive organs (paired testes, seminal vesicles, and preputial glands in males and paired ovaries and uteri in females) were determined.

In female offspring, time to vaginal opening was significantly ($p < 0.001$) increased from 24.6 days in controls to 27.1 days in treated mice. Mating studies in female offspring showed decreased numbers of pregnant females (35% decrease; $p < 0.025$), implantations (12% decrease; $p < 0.05$), and viable fetuses (14% decrease; $p < 0.05$). No treatment-related effects on female body weight or relative weights of reproductive organs were observed. In male offspring, no treatment-related effects were observed in mating studies or on body weights or weights of reproductive organs.

The results indicate that gestational and lactational exposure of BALB/c mouse dams to drinking water containing 353 mg hexavalent chromium/L as potassium dichromate resulted in impaired reproductive development and function in female offspring. Because of the lack of reporting of body weight data over the course of the study, EPA could not identify NOAEL or LOAEL values, expressed in mg hexavalent chromium/kg-day, for this study.

Banu et al., 2008

Banu et al. (2008) investigated the effects of lactational exposure to hexavalent chromium on sexual development of female rat offspring. Groups of 18 lactating Wistar rats were administered drinking water containing 200 mg potassium dichromate (equivalent to 70.6 mg hexavalent chromium/L) on postpartum days 1 through 21. No specific assessments of dams were conducted. Banu et al. (2008) noted that toxic effects in dams were not "significant," although no additional information regarding maternal toxicity or data on body weights or drinking water consumption in dams were reported. As discussed above, exposure of laboratory animals to hexavalent chromium in drinking water may result in decreased body weight and drinking water consumption; thus, in the absence of data on body weight and drinking water consumption in dams, daily doses of hexavalent chromium cannot be accurately estimated for this study. At birth, litters were culled to four female pups per dam. Following weaning on PND 21, pups were separated from dams. Pups (n = 24) were evaluated for the onset of puberty by daily examination for vaginal opening. After the onset of puberty, the time spent in each estrous cycle phase (proestrous, estrous, metestrous, and diestrous) was determined by analysis of vaginal smears (n = 24). On PNDs 21, 45, and 65, pups (n = 24, at each time point) were sacrificed; at each time point, blood was analyzed for hormones (estradiol, progesterone, testosterone, LH, follicle-stimulating hormone [FSH], growth hormone [GH], and prolactin) and ovaries were examined for the number of follicles and follicle development stage (primordial, primary, secondary, and antral).

The onset of puberty was significantly ($p < 0.05$) increased from 33 days in control rats to 55 days in treated rats. Estrous cycle phase was also altered in treated rats, with the time spent in diestrous significantly ($p < 0.05$) increased by approximately 1.4-fold compared with controls (data presented graphically); time spent in other estrous phases was unaffected by treatment. Evaluations of ovaries on PNDs 21 and 45 showed significant ($p < 0.05$) decreases in the numbers of primordial, primary, secondary, and antral follicles in treated rats compared with control rats; on PND 65, the numbers of primordial and primary follicles were also decreased in treated rats. At the 21- and 45-day assessments in treated rats, plasma concentrations of estradiol, progesterone, testosterone, GH, and prolactin were significantly ($p < 0.05$) decreased (by approximately 40–60%) and concentrations of FSH were significantly increased (by approximately 40%), compared with controls. Similar effects were observed at the 65-day

assessment, except that FSH concentrations in treatment and control groups were comparable. Plasma LH concentration was not affected by treatment at any time point.

The results indicate that lactational exposure of Wistar rat dams to drinking water containing 70.6 mg hexavalent chromium/L as potassium dichromate resulted in delayed onset of puberty and follicular development and impaired ovarian steroidogenesis in female offspring; male offspring were not assessed for possible effects on sexual maturation. Because of the lack of reporting of body weight data over the course of the study, EPA could not identify NOAEL or LOAEL values, expressed in mg hexavalent chromium/kg-day, for this study.

4.4. SUMMARY OF HUMAN AND ANIMAL STUDIES BY ROUTES OF EXPOSURE OTHER THAN ORAL

Human exposure to chromium compounds by inhalation has been studied in the chromate production, chrome plating and chrome pigment, ferrochromium production, gold mining, leather tanning, and chrome alloy production industries. Results of occupational epidemiologic studies of chromium-exposed workers have consistently demonstrated excess risks for lung cancer with chromium exposure. Epidemiological studies of chromate production plants in Japan, Great Britain, West Germany, and the United States have revealed a correlation between occupational exposure to chromium (specific form not identified) and lung cancer (Mancuso, 1997; Davies, 1984; Watanabe and Fukuchi, 1975; Frentzel-Beyme, 1983; Langard and Vigander, 1983; Korallus et al., 1982; Alderson et al., 1981; Haguenor et al., 1981; Satoh et al., 1981; Hayes et al., 1979; Hill and Ferguson, 1979; Ohsaki et al., 1978; Sano and Mitohara, 1978; Mancuso, 1975; Enterline, 1974; Taylor, 1966; Todd, 1962; Bidstrup and Case, 1956; Brinton et al., 1952; Bidstrup, 1951; Mancuso and Hueper, 1951; Baejter, 1950a,b; Machle and Gregorius, 1948). Similarly, studies of chrome pigment workers in the United States (Hayes et al., 1989), England (Davies, 1984, 1979, 1978), Norway (Langard and Vigander, 1983; Langard and Norseth, 1975), and in the Netherlands and Germany (Frentzel-Beyme, 1983) have demonstrated an association between occupational chromium exposure and lung cancer. Finally, several studies of the chrome plating industry have demonstrated a positive relationship between cancer and exposure to chromium compounds (Sorahan et al., 1987; Royle, 1975).

Animal data via the inhalation route of exposure are consistent with the findings of human epidemiological studies of hexavalent chromium. Hexavalent chromium compounds were carcinogenic in animal assays producing the following tumor types: lung tumors following inhalation of aerosols of sodium chromate and pyrolized Cr(VI)/Cr(III) oxide mixtures in rats (Glaser et al., 1986), lung tumors following intratracheal administration of sodium dichromate in rats (Steinhoff et al., 1983), intramuscular injection site tumors for various Cr(VI) compounds in Fischer 344 and Bethesda Black rats and in C57BL mice (Furst et al., 1976; Payne, 1960a); intrapleural implant site tumors for various Cr(VI) compounds in Sprague-Dawley and Bethesda Black rats (Hueper and Payne, 1962; Hueper, 1961; Payne, 1960b), and intrabronchial

implantation site tumors for various Cr(VI) compounds in Wistar rats (Levy and Martin, 1983; Laskin et al., 1970; Levy, (as cited in NIOSH, 1975).

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genotoxicity Studies

The mutagenic potential of hexavalent chromium has been studied extensively. Although study results vary with specific test systems, experimental conditions, and the type of hexavalent chromium compound tested, results of in vitro and in vivo studies provide substantial evidence for the mutagenic activity of hexavalent chromium. A general summary of this evidence is provided in Table 4-20. As discussed in detail in Section 4.4.2 (Intracellular Reduction), mutagenicity of hexavalent chromium is mediated through the generation of the highly reactive chromium intermediates penta- and tetravalent chromium, reactive oxygen species, and trivalent chromium formed during the intracellular reduction of hexavalent chromium. These chromium and oxygen species can react with DNA, leading to oxidative DNA damage, chromium-DNA adducts, DNA strand breaks, and chromosomal aberrations.

Table 4-20. Evidence of mutagenicity of hexavalent chromium compounds in experimental systems

Chemical	In vitro studies (nonmammalian cells)		In vitro studies (mammalian cells)			In vivo studies (*Drosophila melanogaster* or mammals)		
	DNA damage	Mutations	DNA damage	Mutations	Chromosomal damage	DNA damage	Mutations	Chromosomal damage
Ammonium chromate	ND	+	ND	ND	ND	ND	ND	ND
Calcium chromate	ND	+	ND	ND	+	ND	+ (D)	ND
Chromic acid	ND	+	ND	ND	+	ND	+ (D)	ND
Potassium chromate	+	+	+	+	+	+ (M)	+ (D) + (M)	+ (M)
Potassium dichromate	+	+	+	+	+	+ (M)	+ (D)	+ (M)
Sodium chromate	ND	+	+	ND	+	ND	ND	ND
Sodium dichromate	ND	+	+	ND	+	+ (M)	+ (D)	ND
Sodium dichromate dihydrate	ND	+	ND	+	ND	ND	ND	+ (M)

+ = positive results; (D) = study in *D. melanogaster*; (M) = study in laboratory mammal; ND = no data identified for this review

4.5.1.1. *Genotoxicity Assays in Experimental Systems*

The mutagenic activity of hexavalent chromium has been demonstrated in numerous studies using both in vitro and in vivo experimental systems. In in vitro test systems (see Tables 4-21 and 4-22 for studies in nonmammalian and mammalian cells, respectively), hexavalent chromium compounds have mostly tested positive for gene mutations (including reverse mutations, frame shift mutations, and base pair substitutions) and DNA damage (including DNA-protein crosslinks) in bacterial cells (*Salmonella typhimurium*, *Escherichia coli*, *Bacillus subtilis*). Reverse mutations were observed in multiple species and strains, including those that are sensitive to frameshift mutagens (*S. typhimurium* TA97, TA98, TA1537, and TA1538), G/C base-pair substitution mutagens (*S. typhimurium* TA100 and TA1535), and A/T base-pair substitution mutagens caused by oxidizing and/or cross-linking agents (*S. typhimurium* TA102; *E. coli* WP2uvrA and WP2uvrA/pKM101). Positive results were also found for forward mutations and mitotic gene conversion in yeast (*Saccharomyces cerevisiae*), and DNA damage (DNA strand breaks, fragmentation, DNA-protein crosslinks, DNA-DNA crosslinks), chromosomal damage (sister chromatid exchanges and chromosomal aberrations), and DNA synthesis inhibition in mammalian cell lines and primary cultures (including primary cultures of human gastric mucosal cells, respiratory tract cells, and lymphocytes).

Table 4-21. In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Reverse mutations	Ammonium chromate	*S. typhimurium* TA97, TA1538, TA98, TA100	+	NS	De Flora et al., 1984
Reverse mutations	Ammonium chromate	*S. typhimurium* TA1537	±	NS	De Flora et al., 1984
Reverse mutations	Ammonium chromate	*S. typhimurium* TA1535	–	–	De Flora et al., 1984
Reverse mutations	Calcium chromate	*S. typhimurium* TA97, TA1538, TA98, TA100	+	NS	De Flora et al., 1984
Reverse mutations	Calcium chromate	*S. typhimurium* TA1537	±	NS	De Flora et al., 1984
Reverse mutations	Calcium chromate	*S. typhimurium* TA1535	–	–	De Flora et al., 1984
Reverse mutations	Calcium chromate	*S. typhimurium* TA98	–	±	Dunkel et al., 1984
Reverse mutations	Calcium chromate	*S. typhimurium* TA100, TA1535, TA1537, TA1538	–	–	Dunkel et al., 1984
Reverse mutations	Calcium chromate	*E. coli* WP2 uvrA	–	±	Dunkel et al., 1984
Reverse mutations	Chromic acid	*S. typhimurium* TA102, TA2638	+	ND	Watanabe et al., 1998
Reverse mutations	Chromic acid	*E. coli*, WP2/pKM101, WP2 uvrA/pKM101	+	ND	Watanabe et al., 1998
Reverse mutations	Chromium trioxide	*S. typhimurium* TA97, TA1538, TA98, TA100	+	NS	De Flora et al., 1984
Reverse mutations	Chromium trioxide	*S. typhimurium* TA1537	±	NS	De Flora et al., 1984
Reverse mutations	Chromium trioxide	*S. typhimurium* TA1535	–	–	De Flora et al., 1984
Reverse mutations	Potassium chromate	*S. typhimurium* TA102	+	ND	Marzin and Phi, 1985
Reverse mutations	Potassium chromate	*S. typhimurium* TA97, TA1538, TA98, TA100	+	NS	De Flora et al., 1984
Reverse mutations	Potassium chromate	*S. typhimurium* TA1537	±	NS	De Flora et al., 1984
Reverse mutations	Potassium chromate	*S. typhimurium* TA1535	–	–	De Flora et al., 1984
Reverse mutations	Potassium chromate	*E. coli* Hs30R	+	ND	Nakamuro et al., 1978
Reverse mutations	Potassium chromate	*E. coli* WP2 hcr- try-, B/rWP2	+ (WP2 hcr)	ND	Kanematsu et al., 1980
Reverse mutations	Potassium chromate	*E. coli* WP2(try-)	+	ND	Venitt and Levy, 1974
Reverse mutations	Potassium chromate	*E. coli* WP2uvrA, CM571	+	ND	Seo and Lee, 1993

Table 4-21. In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Reverse mutations	Potassium dichromate	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	+	+	Zeiger et al., 1992
Reverse mutations	Potassium dichromate	*S. typhimurium* TA102	+	ND	Marzin and Phi, 1985
Reverse mutations	Potassium dichromate	*S. typhimurium* TA100	+	+	Venier et al., 1982
Reverse mutations	Potassium dichromate	*E. coli* WP2 hcr- try-, B/rWP2	+ (WP2 hcr)	ND	Kanematsu et al., 1980
Reverse mutations	Potassium dichromate	*E. coli* Hs30R	+	ND	Nakamuro et al., 1978
Reverse mutations	Potassium dichromate	*E. coli* WP2, WP2uvrA, CM571	+	ND	Nishioka, 1975
Reverse mutations	Potassium dichromate	*E. coli* WP2uvrA, CM571	+	ND	Seo and Lee, 1993
Reverse mutations	Potassium dichromate	*S. cerevisiae* D7	+	ND	Singh, 1983
Reverse mutations	Potassium dichromate	*S. typhimurium* TA98	±	–	Venier et al., 1982
Reverse mutations	Potassium dichromate	*S. typhimurium* TA1538	–	–	Venier et al., 1982
Reverse mutations	Sodium chromate	*E. coli* WP2(try-)	+	ND	Venitt and Levy, 1974
Reverse mutations	Sodium dichromate	*S. typhimurium* TA102, TA2638	+	ND	Watanabe et al., 1998
Reverse mutations	Sodium dichromate	*S. typhimurium* TA102	+	+	Bennicelli et al., 1983
Reverse mutations	Sodium dichromate	*S. typhimurium* TA100	+	–	De Flora, 1978
Reverse mutations	Sodium dichromate	*S. typhimurium* TA97	+	NS	De Flora et al., 1984
Reverse mutations	Sodium dichromate	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984
Reverse mutations	Sodium dichromate	*S. typhimurium* TA1535	–	–	De Flora et al., 1984
Reverse mutations	Sodium dichromate	*E. coli*, WP2/pKM101, WP2 uvrA/pKM101	+	ND	Watanabe et al., 1998
Reverse mutations	Sodium dichromate dihydrate	*S. typhimurium* TA102, TA2638a	+	–	Ryden et al., 2000
Reverse mutations	Sodium dichromate dihydrate	*S. typhimurium* TA100, TA98	+	+	NTP, 2007
Reverse mutations	Sodium dichromate dihydrate	*E. coli*, WP2 uvrA/pKM101	+	+	NTP, 2007
Induction of SOS response	Chromic acid	*E. coli* AB1157, GC2375, UA4202, PQ30	+	ND	Llagostera et al., 1986
Induction of SOS response	Potassium chromate	*E. coli* PQ37, PQ35	+	–	Olivier and Marzin, 1987

Table 4-21. In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Induction of SOS response	Potassium chromate	*E. coli* AB1157, GC2375, UA4202, PQ30	+	ND	Llagostera et al., 1986
Induction of SOS response	Potassium dichromate	*E. coli* AB1157, GC2375, UA4202, PQ30	+	ND	Llagostera et al., 1986
Induction of SOS response	potassium dichromate	*E. coli* PQ37, PQ35	+	–	Olivier and Marzin, 1987
Mutations	Ammonium chromate	*S. typhimurium* TA1978 (rec+), TA1538 (rec -)	+	ND	Gentile et al., 1981
Mutations	Ammonium chromate	*B. subtilis*	+	ND	Gentile et al., 1981
Mutations	Calcium chromate	*S. typhimurium* TA97, TA98, TA100	–	–	Brams et al., 1987
Mutations	Chromic acid	*S. typhimurium* TA1978 (rec+), TA1538 (rec -)	+	ND	Gentile et al., 1981
Mutations	Chromic acid	*B. subtilis*	+	ND	Gentile et al., 1981
Mutations	Potassium chromate	*S. typhimurium* TA98, TA100, TA1537	+	ND	Arlauskas et al., 1985
Mutations	Potassium chromate	*S. typhimurium* TA100	+	ND	Arlauskas et al., 1985
Mutations	Potassium chromate	*E. coli* WP2 uvrA pKm 101	+	ND	Arlauskas et al., 1985
Mutations	Potassium chromate	*B. subtilis* H17	+	ND	Nishioka, 1975
Mutations	Potassium chromate	*S. typhimurium* TA1535, TA1538	–	ND	Arlauskas et al., 1985
Mutations	Potassium dichromate	*S. typhimurium* TA 1535 pSK1002	+	+	Yamamoto et al., 2002
Mutations	Potassium dichromate	*S. typhimurium* TA100, TA1025, TA98	+	ND	Le Curieux et al., 1993
Mutations	Potassium dichromate	*S. typhimurium* TA1978 (rec+), TA1538 (rec-)	+	ND	Gentile et al., 1981
Mutations	Potassium dichromate	*E. coli* WP2uvrA	+	ND	Venier et al., 1987
Mutations	Potassium dichromate	*B. subtilis*	+	ND	Gentile et al., 1981
Mutations	Sodium dichromate	*B. subtilis*	+	ND	Gentile et al., 1981
Mutations	Potassium dichromate	*B. subtilis* NIG45, NIG17	+	ND	Matsui, 1980
Mutations	Potassium dichromate	*B. subtilis* H17	+	ND	Nishioka, 1975

Table 4-21. In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Mutations	Sodium dichromate	*S. typhimurium* TA1978 (rec+), TA1538 (rec -)	+	ND	Gentile et al., 1981
Frame shift mutations	Calcium chromate	*S. typhimurium* TA98, TA1537	+	ND	Haworth et al., 1983
Frame shift mutation	Potassium chromate	*S. typhimurium* TA1537	+	ND	La Velle, 1986
Frame shift mutation	Potassium chromate	*E. coli* 343/358, /415, /435, /477	+	ND	La Velle, 1986
Frame shift mutations	Potassium dichromate	*S. typhimurium* TA97a, TA98	+	+	Tagliari et al., 2004
Frame shift mutations	Potassium dichromate	*S. typhimurium* TA100, TA1537, TA1538	–	ND	Kanematsu et al., 1980
Frame shift mutations	Sodium dichromate	*S. typhimurium* TA97, TA1978	+	ND	Bennicelli et al., 1983
Frame shift mutations	Sodium dichromate	*S. typhimurium* TA1537, TA1538	–	ND	Bennicelli et al., 1983
Frame shift mutations, base pair substitutions	Calcium chromate	*S. typhimurium* TA1537, TA98, TA100	+	+	Petrilli and De Flora, 1977
Frame shift mutations, base pair substitutions	Calcium chromate	*S. typhimurium* TA1535	–	–	Petrilli and De Flora, 1977
Frame shift mutations, base pair substitutions	Chromic acid	*S. typhimurium* TA1537, TA98, TA100	+	+	Petrilli and De Flora, 1977
Frame shift mutations, base pair substitutions	Chromic acid	*S. typhimurium* TA1535	–	–	Petrilli and De Flora, 1977
Frame shift mutations, base pair substitutions	Potassium chromate	*S. typhimurium* TA1537, TA98, TA100	+	+	Petrilli and De Flora, 1977
Frame shift mutations, base pair substitutions	Potassium chromate	*S. typhimurium* TA1535	–	–	Petrilli and De Flora, 1977
Frame shift mutations, base substitutions	Potassium dichromate	*S. typhimurium* TA98 TA100, TA1535, TA1538	+	+	Bianchi et al., 1983
Frame shift mutations, base pair substitutions	Sodium dichromate	*S. typhimurium* TA1537, TA98, TA100	+	+	Petrilli and De Flora, 1977
Frame shift mutations, base pair substitutions	Sodium dichromate	*S. typhimurium* TA1535	–	–	Petrilli and De Flora, 1977
Base pair substitutions	Ammonium chromate	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984; De Flora, 1981
Base pair substitutions	Ammonium chromate	*S. typhimurium* TA1535	–	–	De Flora, 1981

Table 4-21. In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Base pair substitutions	Calcium chromate	*S. typhimurium* TA100, TA1535	+	ND	Haworth et al., 1983
Base pair substitutions	Calcium chromate	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984; De Flora, 1981
Base pair substitutions	Calcium chromate	*S. typhimurium* TA1535	–	–	De Flora, 1981
Base pair substitutions	Chromic acid	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984; De Flora, 1981
Base pair substitutions	Chromic acid	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984; De Flora, 1981
Base pair substitutions	Potassium chromate	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984; De Flora, 1981
Base pair substitutions	Potassium chromate	*S. typhimurium* TA1535	–	–	De Flora, 1981
Base pair substitutions	Potassium dichromate	*S. typhimurium* TA100, TA102	+	+	Tagliari et al., 2004
Base pair substitutions	Potassium dichromate	*S. typhimurium* TA100, TA1535	–	ND	Kanematsu et al., 1980
Base pair substitutions	Sodium dichromate	*S. typhimurium* TA100, TA102, TA92	+	ND	Bennicelli et al., 1983
Base pair substitutions	Sodium dichromate	*S. typhimurium* TA1537, TA1538, TA98, TA100	±	NS	De Flora et al., 1984; De Flora, 1981
Base pair substitutions	Sodium dichromate	*S. typhimurium* TA1535	–	–	De Flora, 1981
Base pair substitutions	Sodium dichromate	*S. typhimurium* TA1535	–	ND	Bennicelli et al., 1983
Reverse mutation, induction of gene conversion	Potassium dichromate	S. *cerevisiae* D7	+	ND	Kharab and Singh, 1985
Forward mutation	Potassium dichromate	*Schizosaccharomyces pombe* 972, h-	±	ND	Bonatti et al., 1976
Mitotic cross-over	Chromic acid	*S. cerevisiae* D7	+	ND	Fukunaga et al., 1982
Mitotic gene conversions	Chromic acid	*S. cerevisiae* D7	+	ND	Singh, 1983; Fukunaga et al., 1982
Mitotic gene conversion, point reverse mutation	Sodium chromate	*S. cerevisiae* D7	+	ND	Bronzetti and Galli, 1989

Table 4-21. In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Mitotic gene conversion at trp5 locus, reverse mutation of ilvl-92 allele	Chromic acid	*S. cerevisiae* D7	+	ND	Vashishat and Vasudeva, 1987
Mitotic gene conversion at trp5 locus, reverse mutation of ilvl-92 allele	Potassium dichromate	*S. cerevisiae* D7	+	ND	Vashishat and Vasudeva, 1987
Induction of disomic and diploid spores	Potassium dichromate	*S. cerevisiae* D1S13	+	ND	Sora et al., 1986
umu gene expression	Potassium dichromate	*S. typhimurium* TA1535	±	–	Nakamura et al., 1987
DNA damage	Potassium dichromate	*E. coli* PQ37	+	ND	Le Curieux et al., 1993
DNA-protein crosslinks	Potassium chromate	*E. coli* DNA	–	ND	Fornace et al., 1981
DNA polymerase arrest	Sodium dichromate	PSV2neo-based plasmid DNA	–	+	Bridgewater et al., 1998, 1994

+ = positive; ± = equivocal or weakly positive; – = negative; ND = no data; NS = not specified

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
DNA damage	Potassium dichromate	Human lymphocytes	+	ND	Blasiak and Kowalik, 2000
DNA damage	Potassium dichromate	Human gastric mucosa	+	ND	Trzeciak et al., 2000
DNA damage	Potassium dichromate	Human peripheral blood lymphocytes	+	ND	Trzeciak et al., 2000
DNA damage	Potassium dichromate	Human lymphocytes, human lymphoblastoid TK-6 cells	+	ND	Cemeli et al., 2003
DNA damage	Sodium dichromate	Human gastric mucosa cells, rat gastric mucosa cells	+	ND	Pool-Zobel et al., 1994
DNA adducts, [^{32}P] postlabeling	Potassium chromate	Calf thymus DNA	–	– (+1 mM H_2O_2)	Adams et al., 1996
DNA fragmentation	Potassium chromate	Human bronchial epithelial cells	+	ND	Fornace et al., 1981
DNA fragmentation	Potassium chromate	Human embryonic lung fibroblasts (IMR-90)	+	ND	Fornace et al., 1981
DNA fragmentation	Potassium chromate	Mouse L1210 leukemia cells	+	ND	Fornace et al., 1981
DNA fragmentation	sodium chromate	Chinese hamster ovary cells	+	ND	Blankenship et al., 1997
DNA strand breaks	Potassium dichromate	Vero kidney fibroblasts, Pam 212 keratinocytes	+	ND	Flores and Perez, 1999
DNA strand breaks	Sodium dichromate	Rat primary lymphocytes	+	ND	Gealy et al., 2007
DNA strand breaks	Sodium dichromate	Rat hepatocytes	+	ND	Gao et al., 1993
DNA strand breaks	Potassium chromate	Human lymphocytes	+	ND	Depault et al., 2006

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
DNA strand breaks	Potassium chromate	Human fibroblast	+	ND	Fornace, 1982
DNA strand breaks	Potassium chromate	Bacteriophage λ DNA	+	+ (+1mM $H_2O_{2)}$	Adams et al., 1996
DNA strand breaks	Sodium dichromate	Rat primary lymphocytes	+	ND	Elia et al., 1994
DNA strand breaks	Potassium dichromate	Human lymphocytes, human gastric mucosa cells	+	ND	Blasiak et al., 1999
DNA-DNA crosslinks	Sodium chromate	Human lung fibroblasts	+	ND	Xu et al., 1996
DNA-protein crosslinks	Potassium chromate	Human embryonic lung fibroblasts (IMR-90)	+	ND	Fornace et al., 1981
DNA-protein crosslinks	Potassium chromate	Human fibroblast	+	ND	Fornace, 1982
DNA-protein crosslinks	Potassium chromate	Chinese hamster cells (V79-UL)	+	ND	Merk et al., 2000
DNA-protein crosslinks	Potassium chromate	Mouse L1210 leukemia cells	+	ND	Fornace et al., 1981
DNA-protein crosslinks	Sodium chromate	Human HL-60 cells	+	ND	Capellmann et al., 1995
Induced DNA methylation	Potassium chromate	Chinese hamster V79 cells (*hpr*$^-$1*gpt*$^+$ transgenic cell line G12)	+ (T)	ND	Klein et al., 2002
Unscheduled DNA synthesis	Sodium dichromate	Rat hepatocytes	+ (T)	ND	Gao et al., 1993
DNA synthesis inhibition	Potassium chromate	HeLa S3 cells	+	ND	Heil and Reifferscheid, 1992
DNA synthesis inhibition	Potassium dichromate	Mouse L cells	+	ND	Nishio and Uyeki, 1985

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
DNA polymerase arrest	Sodium chromate	Human lung fibroblasts	+	ND	Xu et al., 1996
Mutations at the HGPRT locus	Potassium dichromate	Chinese hamster ovary cells (AT3-2)	+	ND	Paschin et al., 1983
Mutations at the HGPRT locus	Potassium dichromate	Chinese hamster cells (V79)	+	ND	Paschin et al., 1983
Forward mutation	Calcium chromate	Mouse lymphoma cells (L5178Y tk^+/tk^-)	+	+	McGregor et al., 1987
Forward mutation	Calcium chromate	Mouse lymphoma cells (L5178Y tk^+)	+	+	Mitchell et al., 1988
Forward mutation	Calcium chromate	Mouse lymphoma cells (L5178Y tk^+)	+	+	Myhr and Caspary, 1988
Forward mutation	Calcium chromate	Mouse lymphoma cells (L5178Y tk^+)	+	+	Oberly et al., 1982
Morphological transformation	Calcium chromate	Syrian hamster embryo cells	+	ND	Elias et al., 1991
Morphological transformation	Sodium chromate dihydrate	Syrian hamster cells	+	ND	DiPaolo and Casto, 1979
Cell transformation	Calcium chromate	Balb/3T3, Syrian hamster embryo, R-MuLV-RE cells	+	ND	Dunkel et al., 1981
Transformations	Potassium chromate	Rat liver epithelial cells	+	ND	Briggs and Briggs, 1988
Chromosomal damage	Calcium chromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Chromosomal damage	Chromic acid	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Chromosomal damage	Potassium chromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Chromosomal damage	Potassium dichromate	Chinese hamster ovary cells	+	ND	Seoane and Dulout, 1999

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Chromosomal damage	Potassium dichromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Chromosomal damage	Potassium dichromate	Chinese hamster ovary cells	+	ND	Majone and Levis, 1979
Chromosomal damage	Sodium chromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Chromosomal damage	Sodium dichromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Chromosomal damage	Sodium dichromate	Chinese hamster ovary cells	+	ND	Majone and Levis, 1979
Chromosome aberrations	Calcium chromate	Chinese hamster lung DON cells	+	ND	Koshi and Iwasaki, 1983; Koshi, 1979
Chromosome aberrations	Calcium chromate	Chinese hamster ovary cells (C3H10T1/2)	+	ND	Sen et al., 1987
Chromosome aberrations	Chromic acid	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Chromosome aberrations	Chromic acid	Mouse mammary FM3A carcinoma cells	+	ND	Umeda and Nishmura, 1979
Chromosome aberrations	Chromic acid	Chinese hamster lung DON cells	+	ND	Koshi and Iwasaki, 1983; Koshi, 1979
Chromosome aberrations	Potassium chromate	Human fibroblasts	+	ND	MacRae et al., 1979
Chromosome aberrations	Potassium chromate	Chinese hamster lung DON cells	+	ND	Koshi and Iwasaki, 1983; Koshi, 1979
Chromosome aberrations	Potassium chromate	Chinese hamster ovary cells	+	ND	MacRae et al., 1979
Chromosome aberrations	Potassium chromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Chromosome aberrations	Potassium chromate	Mouse mammary FM3A carcinoma cells	+	ND	Umeda and Nishmura, 1979
Chromosome aberrations	Potassium dichromate	Human fibroblasts	+	ND	MacRae et al., 1979

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Chromosome aberrations	Potassium dichromate	Chinese hamster ovary cells	+	ND	MacRae et al., 1979
Chromosome aberrations	Potassium dichromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Chromosome aberrations	Potassium dichromate	Mouse mammary FM3A carcinoma cells	+	ND	Umeda and Nishmura, 1979
Chromosome aberrations	Sodium chromate	Human primary bronchial fibroblasts	+	ND	Wise et al., 2004, 2002
Chromosome aberrations	Sodium chromate	Human bronchial fibroblasts (WTHBF-6 cells)	+	ND	Holmes et al., 2006
Chromosome aberrations	Sodium chromate	Human bronchial epithelial cells (BEP2D cells)	+	ND	Wise et al., 2006a
Chromosome aberrations	Sodium chromate	Chinese hamster ovary cells	+	ND	Blankenship et al., 1997
Chromosome aberrations	Sodium chromate	Chinese hamster ovary cells (AA8 (parental), EM9 (XRCC1 mutant), and H9T3	+	ND	Grlickova-Duzevik, 2006
Chromosome aberrations	Sodium dichromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Chromosome and chromatid aberrations	Potassium dichromate	Human lymphocytes	+	ND	Imreh and Radulescu, 1982
Sister chromatid exchanges	Calcium chromate	Human lymphocytes	+	ND	Gomez-Arroyo et al., 1981
Sister chromatid exchanges	Calcium chromate	Chinese hamster lung DON cells	+	ND	Koshi and Iwasaki, 1983; Koshi, 1979
Sister chromatid exchanges	Calcium chromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Sister chromatid exchanges	Chromic acid	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Sister chromatid exchanges	Chromic acid	Chinese hamster cells DON	+	ND	Ohno et al., 1982
Sister chromatid exchanges	Chromic acid	Chinese hamster lung DON cells	+	ND	Koshi and Iwasaki, 1983; Koshi, 1979

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Sister chromatid exchanges	Chromic acid	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Sister chromatid exchanges	Potassium chromate	Human fibroblasts	+	ND	MacRae et al., 1979
Sister chromatid exchanges	Potassium chromate	Chinese hamster lung DON cells	+	ND	Koshi and Iwasaki, 1983; Koshi, 1979
Sister chromatid exchanges	Potassium chromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Sister chromatid exchanges	Potassium chromate	Chinese hamster ovary cells	+	ND	MacRae et al., 1979
Sister chromatid exchanges	Potassium chromate	Chinese hamster cells DON	+	ND	Ohno et al., 1982
Sister chromatid exchanges	Potassium chromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Sister chromatid exchanges	Potassium dichromate	Human lymphocytes	+	ND	Gomez-Arroyo et al., 1981
Sister chromatid exchanges	Potassium dichromate	Human lymphocytes	+	ND	Imreh and Radulescu, 1982
Sister chromatid exchanges	Potassium dichromate	Human fibroblasts	+	ND	MacRae et al., 1979
Sister chromatid exchanges	Potassium dichromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1981
Sister chromatid exchanges	Potassium dichromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Sister chromatid exchanges	Potassium dichromate	Chinese hamster ovary cells	+	ND	Majone and Levis, 1979
Sister chromatid exchanges	Potassium dichromate	Chinese hamster ovary cells	+	ND	MacRae et al., 1979
Sister chromatid exchanges	Potassium dichromate	Chinese hamster cells DON	+	ND	Ohno et al., 1982

Table 4-22. In vitro genotoxicity studies of hexavalent chromium in mammalian cells

Endpoint	Chemical form	Test system	Results		Reference
			Without activation	With activation	
Sister chromatid exchanges	Potassium dichromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Sister chromatid exchanges	Potassium dichromate	Mouse blastocysts	+	ND	Iijima et al., 1983
Sister chromatid exchanges	Sodium chromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Sister chromatid exchanges	Sodium chromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Sister chromatid exchanges	Sodium dichromate	Chinese hamster ovary cells	+	ND	Levis and Majone, 1979
Sister chromatid exchanges	Sodium dichromate	Chinese hamster ovary cells	+	ND	Majone and Levis, 1979
Sister chromatid exchanges	Sodium dichromate	BHK and Chinese hamster ovary cells	+	ND	Bianchi et al., 1980
Disruption of mitosis	Sodium chromate	Human bronchial fibroblasts (WTHBF-6 cells)	+	ND	Wise et al., 2006b

+ = positive; ± = equivocal or weakly positive; – = negative; (T) = toxicity; ND = no data

In in vivo test systems (see Table 4-23), hexavalent chromium compounds have tested positive for mutations in *Drosophila melanogaster* and for DNA damage (DNA-protein crosslinks, DNA strand breaks), mutations (in mice exposed in utero, in mouse germ cells, and in transgenic mice), chromosomal damage (sister chromatid exchanges, chromosomal aberrations, and micronuclei), and DNA synthesis inhibition in rats and mice. The in vivo studies in laboratory mammals have evaluated the mutagenic activity of hexavalent chromium following exposure by the oral, parenteral, inhalation, and intratracheal routes.

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Oral exposures (drinking water): mice						
DNA deletions	Female C57BL/6Jp^{un}/p^{un} mouse offspring	Potassium dichromate was administered in drinking water at concentrations of 0, 62.5, or 125 mg/L at 10.5 to 20.5 days postcoitum (average dose of 12.5 or 25 mg/kg-day). 20-day-old offspring were harvested to visualize eyespots corresponding to DNA deletions in their retinal pigment epithelium (RPE).	+	62.5 mg Cr(VI)/L	Dose-response; no signs of toxicity observed.	Kirpnick-Sobol et al., 2006
Micronuclei	Pregnant Swiss albino mouse bone marrow, dams; fetal liver and peripheral blood cells	Potassium dichromate was administered in drinking water at concentrations of 0, 5, or 10 mg hexavalent chromium/L throughout the duration of pregnancy. Mice were sacrificed on d 18 of pregnancy and bone marrow cells were collected from dams and liver cells were collected from fetuses.	–	10 mg Cr(VI)/L	No effect on PCE/NCE ratio (no cytotoxicity).	De Flora et al., 2006
		Sodium dichromate dihydrate was administered in drinking water at concentrations of 0, 5, or 10 mg hexavalent chromium/L throughout the duration of pregnancy. Mice were sacrificed on d 18 of pregnancy and bone marrow cells were collected. Liver and peripheral blood samples were collected from the fetuses.	–	10 mg Cr(VI)/L		
	BDF_1 male mouse bone marrow and peripheral blood cells	Potassium dichromate was administered in drinking water at 0, 10, or 20 mg hexavalent chromium/L for 20 d.	–	20 mg Cr(VI)/L		
	BDF_1 mouse (male and female) bone marrow or peripheral blood cells	Sodium dichromate dihydrate was administered in drinking water at 0, 5, 50, and 500 mg hexavalent chromium/L for 210 d. Peripheral blood cells were collected on d 0, 14, 28, 56, and 147; bone marrow cells were collected on d 210.	–	500 mg Cr(VI)/L		
Micronuclei	Swiss-Webster mouse bone marrow cells	Potassium dichromate was administered at concentrations of 0, 1, 5, or 20 mg hexavalent chromium/L in drinking water. One set of mice was allowed access to drinking water ad libitum, for 48 hours, while a second group was administered two bolus doses (20 mL/kg) of the same concentrations at 24 and 48 hours before sacrifice.	–	20 mg Cr(VI)/L	No effect on %PCEs.	Mirsalis et al., 1996

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Micronuclei	$B6C3F_1$ (5/group), BALB/c (5/group), and *am3*-C57BL/6 (10/group) male mouse peripheral red blood cells	Sodium dichromate dihydrate was administered in drinking water for 3 mo at concentrations of 0, 62.5, 125, or 250 mg/L (0, 21.8, 43.6, or 87.2 mg hexavalent chromium/L). NTP estimated average daily doses at 0, 2.8, 5.2, or 8.7 mg hexavalent chromium/kg.	±[b] – +	87.2 mg Cr(VI)/L ($B6C3F_1$) 87.2 mg Cr(VI)/L (BALB/c) 43.6 mg Cr(VI)/L (*am3*-C57BL/6)	No effect on PCE/NCE ratio; no clinical signs of toxicity observed.	NTP, 2007
	$B6C3F_1$ mouse (10/sex/group) peripheral red blood cells	Sodium dichromate dihydrate was administered in drinking water for 3 mo at concentrations 0, 62.5, 125, 250, 500, or 1,000 mg/L (0, 21.8, 43.6, 87.2, 174.5, or 349 mg hexavalent chromium/L). NTP estimated daily doses at 0, 3.1, 5.2, 9.1, 15.7, or 27.9 mg hexavalent chromium/kg.	–	349 mg Cr(VI)/L		
Oxidative DNA damage, DNA protein crosslinks	Female SKH-1 hairless mouse forestomach, glandular stomach, and duodenum cells	Sodium dichromate dihydrate was administered in drinking water at concentrations of 0, 5, and 20 mg hexavalent chromium/L for 9 mo. Using reference values for body weight (0.0353 kg) and daily drinking water intake (0.0085 L/d) for female $B6C3F_1$ mice (U.S. EPA, 1988), doses of 1.20 and 4.82 mg hexavalent chromium/kg-d for the 5 and 20 mg hexavalent chromium/L groups, respectively, were estimated. DNA-protein crosslinks and oxidative DNA damage (8-oxo-2'deoxyguanosine) were measured in forestomach, glandular stomach, and duodenum cells.	–	20 mg Cr(VI)/L	No measure of cytotoxicity; no weight changes in mice.	De Flora et al., 2008
Unscheduled DNA synthesis	Fischer 344 rat hepatocytes	Potassium dichromate was administered at concentrations of 0, 1, 5, or 20 mg hexavalent chromium/L in drinking water ad libitum for 48 hours, while a second group was administered single gavage doses (20 mL/kg) at the same concentrations. Hepatocytes were collected from the rat livers and analyzed in the in vivo-in vitro hepatocyte DNA repair assay.	–	20 mg Cr(VI)/L	No measure of cytotoxicity. RDS not determined.	Mirsalis et al., 1996
Oral exposures (drinking water): rats						
DNA-protein crosslinks	Male Fischer 344 rat liver and splenic lymphocytes	Potassium chromate was administered in drinking water for 3 and 6 wks at 100 and 200 ppm hexavalent chromium. Liver and splenic lymphocytes were examined for DNA-protein crosslinks; crosslinks were detected in liver, not lymphocytes.	+	100 mg Cr(VI)/L	No cytotoxicity detected.	Coogan et al., 1991

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Oral exposures (gavage): mice						
DNA damage, comet assay	Swiss albino mouse leukocytes	Potassium dichromate was administered by single gavage doses of 0, 0.59, 1.19, 2.38, 4.75, 9.5, 19, 38, or 76 mg/kg (0, 0.21, 0.42, 0.84, 1.68, 3.37, 6.7, 13.5, or 26.9 mg hexavalent chromium/kg). Samples of whole blood were collected at 24, 48, 72, and 96 hours, and 1 and 2 wks post-treatment for alkaline SCGE comet assay analysis of leukocytes.	+	0.21 mg Cr(VI)/kg	Dose-response from 0.59-9.5 mg/kg. Peak response at 48 h. No cytotoxicity detected (trypan blue).	Devi et al., 2001
DNA damage, comet assay	Swiss albino mouse peripheral lymphocytes	Potassium dichromate was administered by gavage at doses of 0, 25, 50, and 100 mg/kg for 1 day or daily for 5 consecutive days (0, 8.8, 17.7, and 35.4 mg hexavalent chromium/kg). Statistically significant: DNA damage in lymphocytes, and ROS, apoptosis, and suppression of catalase and SOD in liver and not kidney, at 1 and 5 days. No suppression of MDA in liver.	+ (T)	8.8 mg Cr(VI)/kg	Dose-response; apoptosis detected only in liver, not in kidney. No lipid peroxidation.	Wang et al., 2006
DNA damage, comet assay	ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow	Potassium chromate was administered in single gavage doses of 0 or 320 mg/kg (0 or 85.7 mg hexavalent chromium/kg). Cells were collected 3, 8, and 24 hours after treatment and analyzed for DNA damage using the comet assay. DNA damage was found in stomach, colon, liver, kidney, bladder, lung, and brain, but not in bone marrow.	+	85.7 mg Cr(VI)/kg	One dose group. Effects subsided at 24 h; 3 h peak for bladder only. No clinical or microscopic signs of cytotoxicity.	Sekihashi et al., 2001
Micronuclei	BDF_1 male mouse bone marrow cells	Potassium dichromate was given as a single gavage dose of 0 or 50 mg hexavalent chromium/kg.	–	50 mg Cr(VI)/kg	No effect on PCE/NCE ratio (no cytotoxicity).	De Flora et al., 2006
Micronuclei	Male MS/Ae and CD-1 mouse bone marrow cells	Potassium chromate was administered by single gavage doses of 0, 10, 20, 40, 80, 160, or 320 mg/kg (0, 3.5, 7.1, 14.1, 28.3, 56.6, or 113.1 mg hexavalent chromium/kg).	– (T)	113.1 mg Cr(VI)/kg	Negative up to acutely toxic doses.	Shindo et al., 1989
Parenteral exposures: mice						
Mutation	Female C57BL/6J mouse offspring	Potassium chromate was administered by intraperitoneal (i.p.) injection at a dose of 0, 10, or 20 mg/kg on days 8, 9, and 10 of pregnancy in a mammalian spot test (0, 2.7, or 5.4 mg hexavalent chromium/kg). The offsprings' fur was checked for colored spots from wk 2 through 5 after birth.	+	2.7 mg Cr(VI)/kg	Decline in number of surviving offspring with dose.	Knudsen, 1980
Mutation	Male lacZ transgenic Muta™ mouse liver and bone marrow cells	Potassium chromate was administered as an i.p. dose of 0 or 40 mg/kg once a day for 2 consecutive days (0 or 14.1 mg hexavalent chromium/kg). Only one sampling time at day 7 after second treatment. Statistically significant increase in mutation frequency in liver and not bone marrow.	+	14.1 mg Cr(VI)/kg	One dose group. Cytotoxicity not reported.	Itoh and Shimada, 1997

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Mutation	Male lacZ transgenic Muta™ mouse liver and bone marrow cells	Potassium chromate was administered as an i.p. dose of 0 or 40 mg/kg once a day for 2 consecutive days (0 or 14.1 mg hexavalent chromium/kg). Two sampling times (1 and 7 days) after second treatment. Statistically significant increase in mutation frequency in bone marrow on d 1 (not d 7) and in liver on d 7 (not d 1).	+	14.1 mg Cr(VI)/kg	One dose group. Cytotoxicity not reported.	Itoh and Shimada, 1998
Dominant lethality	CBA × C57Bl/6J hybrid male mouse	Potassium dichromate was administered as a single i.p. injection of 0, 0.5, 1.0, 2.0, 10, or 20 mg/kg (0, 0.18, 0.35, 0.70, 3.5, or 7.1 mg hexavalent chromium/kg) or with intraperitoneal injections of 0, 1.0, or 2.0 mg/kg potassium dichromate daily for 21 days (0, 0.35, 0.70 mg hexavalent chromium/kg). Each male was mated with two untreated females for 7 days, and then replaced by two more females every 7 days for 4 consecutive wks. Pregnant dams were sacrificed 12–14 days after conception. The frequency of dominant lethal mutations in male mice was determined based on the postimplantation loss.	+ +	7.1 mg Cr(VI)/kg (acute i.p. injection) 0.7 mg Cr(VI)/kg (repeated i.p. injection)	Statistically significant decrease in embryo survival. Too few doses used to detect dose-response.	Paschin et al., 1982
DNA damage, comet assay	ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow	Potassium chromate was administered as a single i.p. dose of 0 or 120 mg/kg (0 or 32.1 mg hexavalent chromium/kg). Cells were collected 3, 8, and 24 hours after treatment and analyzed for DNA damage using the comet assay. DNA damage was detected in stomach, colon, bladder, lung, and brain, but not in liver, kidney, or bone marrow.	+	32.1 mg Cr(VI)/kg	One dose group. Effects subsided at 24 h; peak at 3 h for bladder, lung, and brain. No clinical or microscopic signs of cytotoxicity.	Sekihashi et al., 2001
DNA damage, comet assay	Male albino mouse liver, kidney, spleen, lung, and brain	Potassium dichromate was administered as a single i.p. dose of 0 or 20 mg hexavalent chromium/kg. Organs were removed and cells were collected for DNA strand break analysis by single-cell gel electrophoresis. DNA damage was detected in liver and kidney (and not in spleen, lung, or brain) at 15 min post-injection; damage back to control levels at 3 h.	+	20 mg Cr(VI)/kg	Same pattern as Cr(V) complexes. Cytotoxicity not reported. DNA damage reduced with deferoxamine	Ueno et al., 2001
Micronuclei	CBA × C57Bl/6J hybrid mouse bone marrow	Potassium dichromate was administered as a single i.p. injection of 0, 1, 5, or 10 mg/kg (0.35, 1.77, or 3.54 mg hexavalent chromium/kg). Bone marrow was sampled 24, 48, and 72 hours after treatment for the micronucleus test.	+	0.35 mg Cr(VI)/kg	Increased response with dose and time; peak at 48 h. No measure of cytotoxicity.	Paschin and Toropzev, 1982
Micronuclei	Slc:ddY mouse bone marrow cells	Potassium chromate was administered by i.p. injection once a day for 2 consecutive days at doses of 0, 30, 40, and 50 mg/kg (0, 10.6, 14.1, or 17.7 mg hexavalent chromium/kg).	+	10.6 mg Cr(VI)/kg	Statistically significant dose-response. %PCEs decreased in two highest doses only.	Itoh and Shimada, 1996

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Micronuclei	NMRI mouse bone marrow	Potassium chromate was administered by 2 i.p. injections with 24 hours between each injection at doses of 0, 12.12, 24.25, or 48.5 mg/kg (0, 3.2, 6.49, or 13.0 mg hexavalent chromium/kg).	+	13 mg Cr(VI)/kg	Statistically significant, dose-related increase. Cytotoxicity not reported.	Wild, 1978
Micronuclei	MS/Ae and CD-1 male mouse bone marrow cells	Potassium chromate was administered by single i.p. doses of 0, 10, 20, 40, or 80 mg/kg (0, 3.5, 7.1, 14.1, or 28.3 mg hexavalent chromium/kg).	+	14.1 mg Cr(VI)/kg	Dose-response observed. %PCEs only decreased at highest dose.	Shindo et al., 1989
Micronuclei	LacZ transgenic Muta™ male mouse peripheral red blood cells	Potassium chromate was administered by an i.p. dose of 0 or 40 mg/kg once a day for 2 consecutive days (0 or 14.1 mg hexavalent chromium/kg).	+	14.1 mg Cr(VI)/kg	One dose group. Cytotoxicity not reported.	Itoh and Shimada, 1997
Micronuclei	MS and ddY mouse bone marrow cells	Potassium chromate was administered by single i.p. doses of 0, 12.5, 25, or 50 mg/kg (0, 4.4, 8.8, or 17.7 mg hexavalent chromium/kg).	+	17.7 mg Cr(VI)/kg	Dose-response observed. Cytotoxicity not reported.	Hayashi et al., 1982
Micronuclei	BALB/c mouse bone marrow	Potassium dichromate was administered as a single i.p. injection at a dose of 0 or 400 µmol (20.8 mg hexavalent chromium/kg).	+ (T)	20.8 mg Cr(VI)/kg	One dose group; significantly decreased %PCEs.	Wronska-Nofer et al., 1999
Micronuclei	Pregnant Swiss albino mouse: bone marrow, dams; fetal liver and peripheral blood cells	Potassium dichromate was administered as a single i.p. injection at 0 or 50 mg hexavalent chromium/kg on day 17 of pregnancy. Mice were sacrificed on day 18 of pregnancy. Liver and peripheral blood samples were collected from the fetuses and bone marrow from the dams.	+	50 mg Cr(VI)/kg	No effect on PCE/NCE ratio (no cytotoxicity).	De Flora et al., 2006
		Sodium dichromate dihydrate was administered as a single i.p. injection at 0 or 50 mg/kg on day 17 of pregnancy. Mice were sacrificed on day 18 of pregnancy and bone marrow cells were collected. Liver and peripheral blood samples were collected from the fetuses.	+	50 mg Cr(VI)/kg		
	BDF_1 male mouse bone marrow cells	Potassium dichromate was administered as single i.p. doses of 0 or 50 mg hexavalent chromium/kg.	+	50 mg Cr(VI)/kg		
Suppressed nuclear DNA synthesis	Mouse tubular renal cells	Potassium dichromate was given as a single i.p. injection at a concentration of 15–30% of the LD_{50} (unspecified) in a thymidine incorporation inhibiting screening system; an intraperitoneal injection of [^{3}H]-thymidine was administered 15 hours later.	+	NS	No measure of cytotoxicity.	Amlacher and Rudolph, 1981

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Parenteral exposures: rats						
DNA damage, comet assay	Sprague-Dawley rat leukocytes	Potassium dichromate was administered i.p. at doses of 2.5, 5, 7.5, and 10 mg/kg-d for 5 days (0, 0.88, 1.77, 2.65, or 3.54 mg hexavalent chromium/kg-d). Whole blood was sampled at 24, 48, 72, and 96 hours after treatment for analysis of leukocytes.	+	0.88 mg Cr(VI)/kg-d	Abstract only.	Patlolla and Tchounwou, 2006
Chromosomal aberrations	Sprague-Dawley rat bone marrow	Potassium dichromate was administered i.p. at doses of 2.5, 5, 7.5, and 10 mg/kg-d for 5 days (0, 0.88, 1.77, 2.65, or 3.54 mg hexavalent chromium/kg-d). Bone marrow cells were harvested at the end of the exposure period and measured for increases in chromosomal aberrations (CAs), micronuclei (MN), and mitotic indices (MI).	+	0.88 mg Cr(VI)/kg-d	Statistically significant CAs and MI with positive dose-response; increases in MN not observed.	Patlolla et al., 2008
DNA-protein crosslinks	Sprague-Dawley male rat lung, liver, and kidney nuclei	Sodium dichromate was given as a single i.p. injection of 20 or 40 mg/kg (7 or 14 mg hexavalent chromium/kg). *No control group was used in this study.* Nuclei from the right renal cortex, the front hepatic lobe, and the whole lung were used for analysis.	+	7 mg Cr(VI)/kg	No measure of cytotoxicity.	Tsapakos et al., 1983
Intratracheal instillation and inhalation exposures: mice						
Mutations	C57BL/6 Big Blue mouse lung, kidney, and liver	Potassium dichromate was given as single doses of 0 or 6.75 mg hexavalent chromium/kg and allowed 4 wks for gene expression. Isolated DNA samples from lung, liver, and kidney tissues were used for LacI gene mutagenesis assay. Mutations were detected in mouse lung and kidney tissue, but not in liver tissue. Depletion of tissue GSH by pretreatment with buthionine sulfoximine decreased the mutagenic response, suggesting that reduced GSH plays a role in producing reactive intermediates during intracellular reduction of chromium (VI).	+	6.75 mg Cr(VI)/kg	No clinical evidence of toxicity at doses ≤ 6.75 mg/kg.	Cheng et al., 2000
Intratracheal instillation and inhalation exposures: rats						
DNA alterations	Sprague-Dawley rat lung and liver	Sodium dichromate was administered as intratracheal instillations at doses of 0 or 0.25 mg/kg for 3 consecutive days (0 or 0.09 mg hexavalent chromium/kg). After the last treatment, lung and livers were removed to analyze for DNA fragmentation, DNA-protein crosslinks, and adducts by [^{32}P] postlabeling. DNA-protein crosslinks, DNA fragmentation, and DNA adducts were detected in lung, but not liver.	+	0.09 mg Cr(VI)/kg	No measure of cytotoxicity. Positive results in lung and not liver.	Izzotti et al., 1998

Table 4-23. In vivo genotoxicity studies of hexavalent chromium in rats and mice

Endpoint	Test system	Test conditions	Results	Dose[a]	Comments	Reference
Chromosomal aberrations, sister chromatid exchange	Sprague-Dawley rat bone marrow cells and peripheral lymphocytes	Rats were exposed to chromium fumes (valence state not specified) generated from a plasma flame sprayer and chromium metal powders at a concentration of 1.84 mg/m^3 for 1 wk (5 hours/d, 5 d/wk) or 0.55 mg/m^3 for 2 mo (5 hours/d, 5 d/wk). Cytogenetic analysis was performed 20 hours, 3 d, 7 d, and 1 mo after the last exposure. Chromosome aberrations and sister chromatid exchanges were detected in rat peripheral lymphocytes but not in bone marrow cells.	+	1.84 mg/m^3 (1-wk inhalation exposure) 0.55 mg/m^3 (2-mo inhalation exposure)	Abstract only.	Koshi et al., 1987

+ = positive; ± = equivocal or weakly positive; – = negative; NS = not specified; (T) = toxicity

[a]Lowest effective dose for positive results, highest dose tested for negative results.

[b]NTP determined this result to be equivocal due to a trend test p-value very nearly significant (p = 0.031; α level = 0.025) and a significant response (p = 0.0193) in the highest dose group of 87.2 mg/L.

Table 4-24. In vivo genotoxicity studies of hexavalent chromium in *D. melanogaster*

Endpoint	Chemical	Test system	Test conditions	Results	Dose[a]	Reference
Gene mutation	Calcium chromate	*D. melanogaster*	24-Hr-old males were fed calcium chromate for 72 hours at doses of 0, 500, or 750 ppm. The males were removed and mated.	+	500 ppm (in diet)	Zimmering et al., 1985
Gene mutation	Chromic acid	*D. melanogaster*	24–48-Hr-old males were treated by intraperitoneal injection with 0, 100, 200, 300, and 400 ppm potassium dichromate or 0, 100, 200, and 300 ppm chromium trioxide. The F2 generation of flies was scored for sex-linked recessive lethal.	+	100 ppm (intraperitoneal injection)	Rodriguez-Arnaiz and Martinez, 1986
Gene mutation (wing somatic mutation)	Chromium oxide	*D. melanogaster*	2–3-D-old larvae were fed potassium chromate or chromium(VI) oxide for 3 d at concentrations of 0, 1, or 5 mM.	+	1 mM (in diet)	Graf and Wurgler, 1996
Gene mutation (white-ivory eye spot test)	Chromium oxide	*D. melanogaster*	2–3-D-old larvae were fed potassium chromate or chromium(VI) oxide for 2 d at concentrations of 0, 1, or 5 mM.	–	5 mM (in diet)	Graf and Wurgler, 1996
Gene mutation	Potassium chromate	*D. melanogaster*	Larvae were fed the test substance in wing spot test at concentrations of 0, 0.1, 0.5, 1.0, and 2.5 mM for the duration of their development. Surviving transheterozygous (mwh/flr^3) and inversion heterozygous (mwh/TM3) flies were used.	+	0.1 mM (in diet)	Amrani et al., 1999
Gene mutation (wing somatic mutation)	Potassium chromate	*D. melanogaster*	2–3-D-old larvae were fed potassium chromate or chromium(VI) oxide for 3 d at concentrations of 0, 1, or 5 mM.	+	1 mM (in diet)	Graf and Wurgler, 1996
Gene mutation	Potassium chromate	*D. melanogaster*	3-D-old larvae were fed potassium chromate for 6 hours at concentrations ranging from 0 to 100 mM or 48 hours at concentrations ranging from 0 to 5.0 mM. Marker-heterozygous and balancer-heterozygous wings from adult flies were then examined in the wing somatic mutation and recombination test (SMART).	+	0.5 mM (48 hours) (in diet) 5 mM (6 hours) (in diet)	Spano et al., 2001
Gene mutation (white-ivory eye spot test)	Potassium chromate	*D. melanogaster*	2–3-D-old larvae were fed potassium chromate or chromium(VI) oxide for 2 d at concentrations of 0, 1, or 5 mM.	–	5 mM (in diet)	Graf and Wurgler, 1996
Gene mutation	Potassium dichromate	*D. melanogaster*	3-D-old transheterozygous larvae were fed potassium dichromate at 0 or 0.5 mM and analyzed for multiple wing hair and flare gene mutations in the Drosophila wing SMART.	+	0.5 mM (in diet)	Kaya et al., 2002

Table 4-24. In vivo genotoxicity studies of hexavalent chromium in *D. melanogaster*

Endpoint	Chemical	Test system	Test conditions	Results	Dose[a]	Reference
Gene mutation	Potassium dichromate	*D. melanogaster*	Larvae were fed the test substance in wing spot test at concentrations of 0, 0.1, 0.5, 1.0, and 2.5 mM for the duration of their development. Surviving transheterozygous (mwh/flr^3) and inversion heterozygous (mwh/TM3) flies were used.	+	0.1 mM (in diet)	Amrani et al., 1999
Gene mutation	Potassium dichromate	*D. melanogaster*	24–48-Hr-old males were treated by intraperitoneal injection with 0, 100, 200, 300, and 400 ppm potassium dichromate or 0, 100, 200, and 300 ppm chromium trioxide. The F2 generation of flies was scored for sex-linked recessive lethal.	+	100 ppm (intraperitoneal injection)	Rodriguez-Arnaiz and Martinez, 1986
Gene mutation	Sodium dichromate	*D. melanogaster*	Larvae were treated on filter papers soaked with sodium dichromate at doses of 1.17 and 2.34 mM for 6 hours and then transferred to vials with substrate. Adult males were checked for wild-type pigmented spots in the eyes.	+	2.34 mM	Rasmuson, 1985

+ = positive; ± = equivocal or weakly positive; – = negative; NS = not specified; (T) = toxicity
[a]Lowest effective dose for positive results, highest dose tested for negative results.

Hexavalent chromium-induced mutagenicity has been demonstrated following oral exposure. Oral exposure studies evaluating the mutagenicity of hexavalent chromium in tissues from the GI tract are of particular relevance in light of the results of the NTP (2008) cancer bioassay showing neoplasms of the oral cavity in rats (at 5.9–7.0 mg hexavalent chromium/kg-day) and of the small intestine in mice (at 2.4–3.1 mg hexavalent chromium/kg-day) administered sodium dichromate dihydrate in drinking water for 2 years. In studies involving gavage administration of hexavalent chromium in mice and rats in vivo, DNA damage has been observed in several tissues, including stomach, colon, liver, lung, kidney, bladder, brain, and peripheral leukocytes (Wang et al., 2006; Devi et al., 2001; Sekihashi et al., 2001; Coogan et al., 1991).

In ddY mice, positive results were reported for DNA damage as measured by the comet assay in the stomach and colon following gavage administration of a single high dose of hexavalent chromium (85.7 mg hexavalent chromium/kg) (Sekihashi et al., 2001). This dose is at least 12-fold greater than chronic dosages associated with oral and GI neoplasms in rats and mice (NTP, 2008), although no concurrent cytotoxicity was found. Data on the potential for DNA damage in cells of the GI tract at lower oral doses (e.g., those in the range of the NTP [2008] bioassay) are not available.

Devi et al. (2001) observed DNA damage via the comet assay in mouse leukocytes following an oral dose as low as 0.21 mg/kg, an effect that increased with dose up to 9.5 mg/kg and did not cause a decrease in cell viability. Similarly, Wang et al. (2006) found a dose-dependent increase in DNA damage in peripheral lymphocytes using the comet assay that was found to persist for 5 days post-exposure and was accompanied by a significant increase in reactive oxygen species and apoptosis in the liver. Sekihashi et al. (2001) found comet damage in mouse stomach, colon, liver, kidney, bladder, lung, and brain following a single gavage dose of 85.7 mg/kg. These effects were not accompanied by cytotoxicity, although it is unknown whether a response to dose would have occurred.

Three drinking water exposure studies of hexavalent chromium in mice and rats have yielded positive results for the induction of chromosomal damage. Coogan et al. (1991) observed DNA-protein crosslinks in rat liver following 3- and 6-week exposures that were not accompanied by cytotoxicity. In another drinking water study in a strain of mice containing a mutation allowing for visual representation of chromosome deletions in the form of eye spots in the offspring of exposed pregnant females, exposures to 62.5 mg/L of hexavalent chromium from 10.5 to 20.5 days postcoitum resulted in a significant level of DNA deletions in 20-day old offspring that increased with dose and was not accompanied by cytotoxicity (Kirpnick-Sobol et al., 2006). Statistically significant increases in chromosomal damage (as indicated by micronuclei formation) with a significant dose-response was observed in peripheral RBCs of one strain of mice (*am3*-C57BL/6) exposed to ≥ 43.6 mg hexavalent chromium/kg-day as sodium dichromate dihydrate in drinking water for 3 months, but not in BALB/c mice at daily doses up

to 87.2 mg hexavalent chromium/kg-day (NTP, 2007). The $B6C3F_1$ strain of mice used in the 2-year NTP bioassay (NTP, 2008) was also tested for micronucleus formation; the first test was negative, but the second test showed a nearly significant positive trend for micronucleus formation. These results were considered equivocal overall because the trend test p-value of 0.031 was close in value to the designated alpha level of 0.025 used in this study (compared to the typical alpha level of 0.05).

Other studies have reported negative results in bone marrow or peripheral blood cells following oral exposures (NTP, 2007; De Flora et al., 2006; Mirsalis et al., 1996; Shindo et al., 1989). One study investigated tissues in mice identified by the NTP bioassay (2008) as having significant hexavalent chromium-induced tumors and reported negative results for oxidative DNA damage and DNA-protein crosslinks in cells of the forestomach, glandular stomach, and duodenum of female SKH-1 mice administered drinking water containing 5 or 20 mg hexavalent chromium/L (approximately equivalent to 1.20 and 4.82 mg hexavalent chromium/kg-day, respectively) as sodium dichromate dihydrate for 9 months (De Flora et al., 2008). It is worth noting the absence of positive findings in De Flora et al. (2008) given that the highest dose evaluated in this study is slightly less than chronic dosages associated with neoplasms of the oral cavity in rats (5.9–7.0 mg hexavalent chromium/kg-day), and slightly greater than those associated with neoplasms of the small intestine in mice (2.4–3.1 mg hexavalent chromium/kg-day) (NTP, 2008). No oral exposure studies on the potential clastogenic activity of hexavalent chromium in rat tumor target tissue (oral mucosa) were identified. Although the NTP (2007) 3-month drinking water study evaluated micronuclei formation in peripheral RBCs of mice (with positive results in the *am3*-C57BL/6 strain and equivocal results in the $B6C3F_1$ strain), mutagenic effects of hexavalent chromium exposure in GI tissues were not evaluated in this study.

Results of parenteral exposure studies are uniformly positive for hexavalent chromium-induced mutagenicity. Following parenteral exposure, DNA damage has been observed in numerous tissues, including peripheral lymphocytes, stomach, colon, liver, kidney, bladder, lung, and brain (Patlolla and Tchounwou, 2006; Sekihashi et al., 2001; Ueno et al., 2001); mutations have been observed in liver (Knudsen, 1980); and chromosomal damage (micronuclei) has been observed in peripheral RBCs and bone marrow (De Flora et al., 2006; Itoh and Shimada, 1997; Shindo et al., 1989; Hayashi et al., 1982; Wild, 1978).

Mutagenic activity of hexavalent chromium has also been demonstrated in lung cells of animals following intratracheal exposure. DNA damage (DNA fragmentation, DNA-protein crosslinks, and DNA adducts) was reported in lung cells of Sprague-Dawley rats administered 0.09 mg hexavalent chromium/kg by intratracheal instillation for 3 days (Izzotti et al., 1998) and mutations were reported in lung cells of C57BL/6 mice administered a single intratracheal dose of 7.65 mg hexavalent chromium/kg. Results of these studies are relevant to occupational exposure studies showing increased respiratory tract cancers in hexavalent chromium workers

(see Section 4.4.1.2). No inhalation or intratracheal exposure studies on the potential clastogenic activity of hexavalent chromium in respiratory tract cells were identified. Chromosomal damage (chromosome aberrations and sister chromatid exchange) was observed in peripheral lymphocytes, but not bone marrow, of Sprague-Dawley rats exposed to chromium fumes for 1 week (1.84 mg/m^3) or 2 months (0.55 mg/m^3) (Koshi et al., 1987).

4.5.1.2. *Genotoxicity Studies in Humans*

In addition to mutagenicity evaluations in experimental systems, several studies have evaluated mutagenicity in humans occupationally exposed to hexavalent chromium; experimental details and citations are summarized in Table 4-25. Data from available mutagenicity studies in exposed workers are limited to assessments of tissues with easy accessibility (e.g., circulating lymphocytes and buccal and nasal mucosal cells). Data on mutagenicity in cancer target tissues (e.g., lung and GI tract) are not available. Available data provide some evidence of hexavalent chromium-induced mutagenicity in occupationally exposed humans, although results of studies in workers have yielded mixed results. In general, associations between hexavalent chromium exposure and mutagenicity in workers are uncertain because exposure levels were often not quantified or estimated, past exposure history was not well characterized in all studies, small numbers of workers were evaluated, and/or workers were potentially co-exposed to other compounds with mutagenic activity.

Table 4-25. In vivo genotoxicity studies in humans exposed to hexavalent chromium

Endpoint	Exposure type (chemical form)	Cell type	Test conditions	Results	Exposure level[a]	Reference
DNA strand breaks	Occupational, chromium plating (chromic acid)	Human peripheral lymphocytes	Nineteen chromium plating workers in Italy (mean employment of 6.3 yrs) and two groups of control subjects (18 hospital workers and 20 university personnel) gave pre- and postshift urine samples and blood samples for analysis in the comet assay. Duration of employment ranged from 4 mo to 14 yrs with a mean duration of 6.3 yrs. Mean chromium concentrations in urine were determined to be 5.29 µg/g creatinine (preshift) and 7.31 µg/g creatinine (postshift). Mean erythrocyte and lymphocyte concentrations in the exposed workers were 4.94 µg/L and 50.3 µg/10^{12}cells, respectively. Air concentrations of chromium were not reported.	+	NS	Gambelunghe et al., 2003
DNA strand breaks, hydroxylation of deoxyguanosine	Occupational, production of dichromate (included exposure to chromic acid, potassium dichromate and sodium dichromate)	Human peripheral lymphocytes	Urine and blood samples were taken from 10 exposed workers and 10 nonexposed workers at the end of a workweek at a bichromate production plant in England. The mean duration of exposure was 15 yrs. Chromium concentrations in the factory ranged from 0.001 to 0.055 mg hexavalent chromium/m^3 (obtained from personal and area samplers). Mean chromium concentrations in urine (5.97 µg/g creatinine), whole blood (5.5 µg/L), plasma (2.8 µg/L), and lymphocytes (1.01 µg/10^{10} cells) of exposed workers were significantly higher than in nonexposed workers.	–	0.001–0.055 mg hexavalent chromium/m^3 (measured exposure range)	Gao et al., 1994
DNA-protein crosslinks	Experimental oral exposure (potassium dichromate)	Human peripheral lymphocytes	Four adult volunteers ingested a single bolus dose of 5,000 µg hexavalent chromium as potassium dichromate (approximately equivalent to 71 µg hexavalent chromium/kg, assuming a body weight of 70 kg). Blood samples were collected at 0, 60, 120, 180, and 240 mins after ingestion. Preingestion background DNA-protein crosslink levels for each individual served as the controls.	–	71 µg hexavalent chromium/kg	Kuykendall et al., 1996
Chromosome aberrations, sister chromatid exchanges	Occupational, chromium electroplating (chemical not specified)	Human peripheral lymphocytes	Blood from seven chromium electroplating workers at a Chinese electroplating facility (mean employment period of 12.8 yrs) and 10 control subjects were analyzed. Air samples from the electroplating room were collected, along with stool and hair samples to determine exposure. The mean chromium (total) air concentration (by random air collection) was 8.1 µg/mm^3, the mean chromium concentration in stool samples was 8.5 µg/g stool, and the mean chromium concentration in hair was 35.68 µg/g. The valence of chromium that workers were exposed to was unspecified.	+	8.1 µg chromium/mm^{3b}	Deng et al., 1988

Table 4-25. In vivo genotoxicity studies in humans exposed to hexavalent chromium

Endpoint	Exposure type (chemical form)	Cell type	Test conditions	Results	Exposure level[a]	Reference
Sister chromatid exchanges	Occupational, chromium electroplating (chemical not specified)	Human whole blood cells	Thirty-five chromium electroplating factory workers employed at three electroplating plants in Tawain and 35 control subjects gave blood samples to analyze the frequency of sister chromatid exchange. Exposure duration ranged from 2 to 14 yrs with a mean of 6.5 yrs. Mean chromium exposure (determined by personal monitoring samplers) was 5.99 mg hexavalent chromium/m^3. The mean urinary chromium concentration of the exposed workers was 3.67 µg/g creatinine.	+	5.99 mg hexavalent chromium/m^3	Wu et al., 2001
Chromosomal aberrations, sister chromatid exchanges	Occupational, chromium plating (chromic acid)	Human peripheral lymphocytes	Thirty-eight male chromium plating factory workers in Italy were examined for urinary concentrations of chromium and chromosomal aberrations and sister chromatid exchanges. Chromium exposure levels were not reported. There were 35 unexposed control individuals.	+	NS	Sarto et al., 1982
Sister chromatid exchanges	Occupational, chromium plating (chromic acid fumes)	Human peripheral lymphocytes	The frequency of sister chromatid exchanges was determined in lymphocytes from 12 chromium plating workers in Italy and 10 control subjects. Exposure durations ranged from 0.5 to 18 yrs (mean exposure duration was not reported). Hexavalent chromium exposure levels and blood concentrations were not reported.	+	NS	Stella et al., 1982
Sister chromatid exchanges	Occupational, chromium electroplating (chemical not specified)	Human peripheral lymphocytes	Thirty-five chromium electroplating factory workers in Taiwan and 35 control subjects (matched for age and gender) gave blood samples to determine sister chromatid exchange frequency. The mean duration of employment was 6.5 yrs. Exposure concentrations were not reported.	+	NS	Wu et al., 2000
Chromosomal aberrations, sister chromatid exchanges	Occupational, chromium plating (chemical not specified)	Human peripheral lymphocytes and buccal mucosal cells	Blood samples and buccal mucosal cells from 15 Bulgarian chromium platers occupationally exposed were taken; exposure was estimated with personal air samplers and in urine samples. Control subjects were matched with exposed individuals. Duration of exposure ranged from 2 to >20 yrs; mean duration of exposure was not reported. Mean air concentration of total chromium was 0.0075 mg chromium/m^3 in the low-exposure group and 0.0249 mg chromium/m^3 in the high-exposure group (number of workers in each exposure group was not reported). Mean concentrations of chromium in urine were 18.63 µg/L (low) and 104.22 µg/L (high)	–	Results reported for combined groups (0.0075 and 0.0249 mg chromium/m^3)	Benova et al., 2002

Table 4-25. In vivo genotoxicity studies in humans exposed to hexavalent chromium

Endpoint	Exposure type (chemical form)	Cell type	Test conditions	Results	Exposure level[a]	Reference
Sister chromatid exchanges	Occupational, chromium plating (chemical not specified)	Human peripheral lymphocytes	Venous blood and urine sample were collected from 12 male chromium platers in Japan over a 5-yr period. No control subjects were used in this study. Employment duration ranged from 6.6 to 25.1 yrs, with mean employment duration of 15.5 yrs. Exposure concentrations were not reported. Urinary chromium concentrations ranged from 1.2 to 57.0 µg/g with a mean urinary chromium concentration of 17.9 µg/g creatinine. Sister chromatid exchange frequency in lymphocytes was determined in blood-urine paired samples.	–	NS	Nagaya et al., 1991
Sister chromatid exchanges	Occupational, chromium plating (chemical not specified)	Human peripheral lymphocytes	Venous blood and urine sample were collected from 24 male chromium platers in Japan and 24 control subjects. Duration of employment ranged from 0.5 to 30.5 yrs with a mean employment of 11.6 yrs. Exposure concentrations were not reported. The mean concentration of chromium in the urine was 13.1 µg/L.	–	NS	Nagaya, 1986
Micronuclei	Occupational, chromium electroplating (chemical not specified)	Human peripheral lymphocytes	Forty electroplating workers in Bulgaria and 18 control subjects gave blood samples to analyze for the frequency of micronuclei. The workers were split into two groups based on levels of exposure. Mean air chromium (total) concentrations were 43 and 83 µg/m^3 in the low- and high-exposure groups, respectively. Duration of employment ranged from 4 to 25 yrs with mean durations of 10.44 and 11.63 yrs in the low- and high-exposure groups, respectively. Mean chromium concentrations in erythrocytes and urine of the low-exposure group were 4.31 and 3.97 µg/L, respectively. The mean chromium concentrations in erythrocytes and urine of the high-exposure group were 8.4 and 5.0 µg/L, respectively.	+	0.043 and 0.083 mg chromium/m^3	Vaglenov et al., 1999
Micronuclei	Occupational, chromium plating (chemical not specified)	Human peripheral lymphocytes and buccal mucosal cells	Blood samples and buccal mucosal cells from 15 Bulgarian chromium platers occupationally exposed were taken. Exposure was estimated with personal air samplers and in urine samples. Control subjects were matched with exposed individuals. Duration of exposure ranged from 2 to >20 yrs; mean duration of exposure was not reported. Mean air concentration of total chromium was 0.0075 mg chromium/m^3 in the low-exposure group and 0.0249 mg chromium/m^3 in the high-exposure group. Mean concentrations of chromium in urine were 18.63 (low) and 104.22 µg/L (high).	+	Positive results reported for combined groups (0.0075 and 0.0249 mg chromium/m^3)	Benova et al., 2002

Table 4-25. In vivo genotoxicity studies in humans exposed to hexavalent chromium

Endpoint	Exposure type (chemical form)	Cell type	Test conditions	Results	Exposure level[a]	Reference
Micronuclei	Occupational, chromium plating (chromic acid)	Human buccal and nasal cells	Sixteen exposed Italian electroplating factory workers and 27 unexposed control subjects gave samples of exfoliated buccal and nasal swabs. Duration of exposure ranged from 0.5 to 23 yrs with a mean duration of 8 yrs. Urine samples were collected at the end of work days to determine chromium exposure. Urinary chromium concentrations ranged from 2.5 to 88 µg/g creatinine; the mean urinary chromium concentration was not reported. Chromium levels in air were not determined.	–	NS	Sarto et al., 1990

[a]All exposure levels associated with positive results, highest exposure level for negative results.
[b]The exposure level of 8.1 µg chromium/mm^3 is as reported by Deng et al. (1988); however, this appears to be a reporting error, as this concentration is equivalent to 8,100,000 mg chromium/m^3.

+ = positive; – = negative; NS = not specified

In a comet assay in Italian chrome platers, positive results were reported for DNA strand breaks in peripheral lymphocytes; although urine chromium concentrations were determined, hexavalent chromium exposure levels were not reported (Gambelunghe et al., 2003). However, no DNA damage was observed in peripheral lymphocytes in dichromate production workers exposed to 0.001–0.055 mg hexavalent chromium/m^3 (Gao et al., 1994) or in volunteers ingesting single oral doses of 71 µg hexavalent chromium/kg (Kuykendall et al., 1996). In chrome electroplaters, chromosome aberrations and sister chromatid exchanges were observed in whole blood of workers exposed to relatively high concentrations estimated at 5.99 mg hexavalent chromium/m^3 (Wu et al., 2001). However, chromosome aberrations and sister chromatid exchanges in peripheral lymphocytes from chrome platers were not observed at lower exposure levels (0.0075 and 0.0249 mg chromium[total]/m^3) (Benova et al., 2002). Other studies reporting positive (Wu et al., 2000; Sarto et al., 1982; Stella et al., 1982) or negative (Nagaya et al., 1991; Nagaya, 1986) results for chromosome aberrations or sister chromatid exchanges in peripheral lymphocytes of workers did not report hexavalent chromium exposure levels. Micronuclei formation in peripheral lymphocytes was also observed in chrome platers at exposure levels of 0.043–0.083 mg chromium(total)/m^3 (Vaglenov et al., 1999) and 0.0075–0.0249 mg chromium(total)/m^3 (Benova et al., 2002). In buccal mucosal cells collected from chrome platers, micronuclei formation was increased at exposure levels of 0.0075–0.0249 mg chromium(total)/m^3, although chromosome aberrations and sister chromatid exchanges were not observed (Benova et al., 2002). Sarto et al. (1990) reported negative results for micronuclei in buccal and nasal cells of chrome platers, but exposure levels were not reported.

In summary, results of available studies in hexavalent chromium-exposed workers provide some evidence of the mutagenic activity of hexavalent chromium in occupationally exposed humans, but results have not been consistent across studies and endpoints. For example, associations with increased micronuclei in peripheral lymphocytes or buccal mucosal cells have been reported in chrome platers at estimated exposure levels as low as 0.0075–0.0249 mg chromium(total)/m^3 (Benova et al., 2002; Vaglenov et al., 1999), although chromosome aberrations and sister chromatid exchanges were not observed (Benova et al., 2002). In contrast, increased frequencies of chromosome aberrations and sister chromatid exchanges were observed in another group of chrome platers exposed to higher concentrations estimated at 5.99 hexavalent chromium/m^3 (Wu et al., 2001).

4.5.2. Intracellular Reduction

The mutagenic effects of hexavalent chromium are contingent upon its reduction within the cell. Extracellularly, soluble hexavalent chromium exists as a chromate oxyanion. The tetrahedral arrangement of the oxygen groups makes it structurally similar to phosphate and sulfate, allowing it to easily be taken up by the nonspecific phosphate/sulfate anionic transporters and cross the cell membrane (Zhitkovich, 2005). This method of cellular uptake also allows an

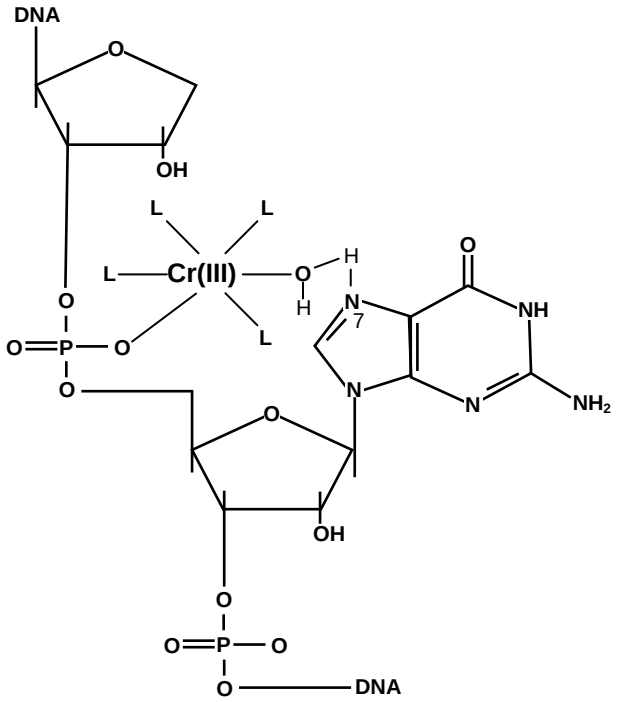
accumulation of chromium in the cell at concentrations much higher than that found extracellularly (Zhang et al., 2002). Chromium in its hexavalent state is thermodynamically stable in pure water, and is not reactive with DNA at physiological concentrations. However, hexavalent chromium is a strong oxidizer, and once inside the cell, it can undergo rapid reduction. This is most often mediated by the nonenzymatic reductants ascorbate (vitamin C) and low molecular weight thiols including GSH and cysteine. Other potential reductants include cytochrome P450 reductase, NAD(P)H-dependent flavoenzymes, and mitochondrial electron transport complexes (O'Brien et al., 2003; Sugden and Stearns, 2000; Standeven and Wetterhahn, 1989).

The hexavalent chromium-reductant substrate complexes that are formed upon intracellular interaction of hexavalent chromium with these reductants are considered the first step in the reduction process, although the actual mechanisms of how these reactions proceed are unknown (Levina and Lay, 2005). There are two theorized pathways for the intracellular reduction of hexavalent chromium. When reductants are present in abundance, the process can occur with a two-electron reduction to tetravalent chromium, immediately followed by a one-electron reduction to trivalent chromium. If lower levels of reductants are available, the first step of this process will occur as two distinct one-electron transfers, producing the intermediates, pentavalent and tetravalent chromium, and ultimately trivalent chromium (O'Brien et al., 2003). Either process can produce oxidative states of chromium localized within the cell that are able to damage DNA directly, forming DNA adducts and subsequent DNA breakage. These chromium species can also indirectly cause genetic damage via associated radical species derived from the reductants that can be involved in secondary DNA damage (Sugden and Stearns, 2000) and disruption of DNA replication.

Final reduction product: trivalent chromium. Trivalent chromium is the ultimate product of hexavalent chromium reduction within the cell. It contains six coordination sites, allowing it to form stable complexes with amino acids, proteins, RNA, and DNA. In vitro studies of the kinetics of chromium−DNA binding have shown that most of the DNA binding occurs within 1 hour of incubation (Quievryn et al., 2003). When hexavalent chromium is reduced by ascorbate or cysteine in the presence of the trivalent chromium chelator, EDTA, the mutagenic response is all but eliminated and very little chromium−DNA binding is detected, indicating that the trivalent state is the most DNA reactive of all the valence states of chromium (O'Brien et al., 2003; Quievryn et al., 2003; Zhitkovich et al., 2001). Several types of chromium−DNA adducts have been detected following the intracellular reduction of hexavalent to trivalent chromium.

DNA−peptide/amino acid ligand−trivalent chromium crosslinks. Trivalent chromium can form ternary DNA crosslinks with GSH, ascorbate, cysteine, and histidine. Although the ascorbate−trivalent chromium−DNA adducts are recovered less frequently in vitro due to the low concentrations of vitamin C present in commonly used tissue culture media (Zhitkovich, 2005),

these adducts have been shown to be the most mutagenic of all the ternary adducts (Quievryn et al., 2003). These ternary adducts form by the attachment of trivalent chromium (in a binary complex with the ligand) to phosphate groups in DNA (Zhitkovich et al., 1995), primarily through coordinate covalent binding or electrostatic/ionic interactions (O'Brien et al., 2003) (Figure 4-1). They have been detected in vitro in Chinese hamster ovary cells following exposure to hexavalent chromium, and account for up to 50% of all chromium−DNA adducts. The ternary adducts have been found to cause mutagenic and replication-blocking lesions in human fibroblasts in vitro (Quievryn et al., 2003; Voitkun et al., 1998).



Hexavalent chromium, when reduced intracellularly to trivalent chromium, can form ternary DNA crosslinks with the peptide or amino acid ligand (L) involved in the reduction. Here, chromium(III) directly coordinates to the 5'-phosphate in the DNA backbone and forms a hydrogen bond with the N-7 of deoxyguanosine.

Source: Zhitkovich (2005).

Figure 4-1. Ternary DNA adduct formation by chromium.

DNA−trivalent chromium crosslinks. Reduction of hexavalent chromium in vitro produces a large proportion of binary trivalent chromium−DNA adducts, but these have not been detected in vivo. It has been theorized that the formation of the ternary adducts described above occurs far more frequently due to the high concentration of ligands capable of complexing with trivalent chromium before it can bind to DNA (Zhitkovich, 2005). In addition, these adducts

have been found to be less mutagenic than the ternary adducts in vitro (Quievryn et al., 2003; Zhitkovich et al., 2001).

DNA−protein crosslinks. These bulky lesions have been detected in hexavalent chromium-treated cells in vitro in Chinese hamster ovary cells (Costa, 1991) and in vivo in chick embryos (Hamilton and Wetterhahn, 1986). They are not detected in the presence of the trivalent chromium chelator, EDTA, indicating that trivalent chromium is the species involved in their formation (Miller and Costa, 1989). It has been recently shown that the mechanism forming DNA−protein crosslinks induced by hexavalent chromium requires intracellular reduction to trivalent chromium, formation of DNA−trivalent chromium adducts, and subsequent capture of proteins by the DNA bound to trivalent chromium (Macfie et al., 2010). Tests for the mutagenicity of these crosslinks have proved inconclusive (reviewed in Macfie et al., 2010), but the bulkiness of these lesions indicates the potential for genotoxicity resulting from replication fork stalling (Costa, 1991).

DNA−DNA crosslinks. These inter- or intra-strand DNA crosslinks are likely formed by oligomers of trivalent chromium. They have been detected following hexavalent chromium exposure, although only when the reductants are ascorbate or cysteine, and not GSH (Zhitkovich, 2005). However, these adducts have only been detected in vitro and are not expected to form in significant amounts in vivo; the high intracellular concentrations of ligands available to form complexes with trivalent chromium make it unlikely that these oligomers would have a chance to form (Salnikow and Zhitkovich, 2008).

Repair of chromium−DNA adducts. Repair processes have been shown to be effectively carried out by excision repair (ER), a DNA repair mechanism responsible for removal of bulky DNA lesions. Exposing nucleotide excision repair (NER)-deficient human cells to hexavalent chromium was shown to induce apoptosis and clonogenic cell death. The most efficient substrates for this repair process are lesions that create major distortions in the DNA structure. Chromium−DNA adducts do not create major helix distortions, but their bulkiness makes them adequate substrates for NER, although they are less efficiently removed than optimal NER substrates such as UV light-induced lesions (Reynolds et al., 2004). Interestingly, it has also been shown that proficient ER systems, including both nucleotide and base excision repair (NER and BER), are involved in genomic instability resulting from hexavalent chromium exposure. In a study by Brooks et al. (2008), cell lines deficient in these repair mechanisms were protected from hexavalent chromium-induced chromosomal instability.

Another closely related repair mechanism, mismatch repair (MMR), is responsible for the correction of errors in DNA replication. MMR enzymes recognize misincorporated bases during DNA replication and homologous recombination, and repair single base mispairings and small insertions or deletions. However, MMR has also been shown to be a causative factor in many of the toxic and genotoxic effects of hexavalent chromium, when processing the repair of the bulky lesions formed by chromium lead to the formation of DNA double-strand breaks (Peterson-Roth

et al., 2005). In this study, mouse and human cell lines deficient in MMR exposed to hexavalent chromium had greatly increased clonogenic survival due to a diminished apoptotic response as compared to MMR-proficient cells. The apoptotic response in the MMR-proficient cells was preceded by a significant induction of DNA double-strand breaks, indicated by an increased formation of gamma-H2AX foci. These discrete foci form when phosphorylation of this histone H2A variant occurs in response to DNA double-strand breaks, and can be visualized and quantified by immunofluorescence. This increase in gamma-H2AX foci was not detected at significant levels until 6 hours postexposure to hexavalent chromium, suggesting that the DNA double-strand breaks were not induced directly by hexavalent chromium, but rather from processing of the damaged DNA. These foci also co-localized with cyclin B1 staining, indicating the breaks occurred in the G2 phase of the cell cycle and providing evidence that passage through S phase, where MMR would be taking place, was necessary for the induction of this damage (Salnikow and Zhitkovich, 2008). The mechanism of this toxic response mediated by MMR proteins is unknown, but has been theorized to involve the futile repair of damaged bases or the initiation of a stress response (Peterson-Roth et al., 2005).

Cellular effects. Genomic instability, defined as an increased rate of acquisition of alterations in the genome, is a hallmark of tumorigenesis and may be instrumental in hexavalent chromium-induced carcinogenicity. The loss of MMR function leads to an unstable mutator phenotype, in which replication errors, particularly those occurring in simple nucleotide repeat sequences known as microsatellites, are not corrected, leading to an increase in mutation frequency (Loeb et al., 2008). Further, chromosomal instability has been demonstrated in human lung cells in vitro exposed to particulate hexavalent chromium. Following chronic exposures, Holmes et al. (2010) found concentration- and time-dependent increases in aneuploidy as the result of centrosome amplification and spindle assembly checkpoint bypass. Thus, genomic instability may result after prolonged exposure to hexavalent chromium.

As mentioned above, apoptosis, or programmed cell death, has been observed in cells exposed to hexavalent chromium as a response to extensive DNA damage that cannot be adequately repaired by the cell. Ye et al. (1999) found hexavalent chromium induced apoptosis in human lung epithelial cells exposed to doses ranging from 75 to 300 μM in vitro; the authors theorized that this response involved reactive oxygen species formed both directly during the process of hexavalent chromium reduction and indirectly through the induction of p53. Similarly, Wang et al. (2006) measured significant levels of apoptosis in liver and not kidney in mice exposed to daily gavage doses of 0, 25, 50, or 100 mg/kg hexavalent chromium for 1 or 5 days. The apoptosis was accompanied by an increase in ROS and dose-dependent increases in DNA damage, SOD, and catalase. Flores and Perez (1999), using doses close to the IC_{50} values, observed apoptosis concurrent with DNA interstrand crosslinks and DNA single-strand breaks in murine keratinocytes transformed with the H-ras oncogene. These studies indicate that multiple

mechanisms induced by hexavalent chromium exposure, including oxidative stress and DNA binding, can lead to cell death.

In addition to the effects involving DNA repair mechanisms is the finding that hexavalent chromium, after intracellular reduction to the +3 oxidation state, can interfere with normal DNA replication and transcription processes. Intracellular trivalent chromium has been shown to inhibit the enzymatic activity of DNA polymerases, simultaneously increasing the rate of replication and the processivity of the DNA polymerase, thereby decreasing its fidelity and causing more frequent errors, with a dose-dependent increase in mutation frequency in vitro (Snow, 1991). There can also be replication arrest as a result of the bulky chromium–DNA lesions, creating a physical obstruction to the progression of DNA polymerases (Bridgewater et al., 1998). These effects were recently confirmed in a study utilizing the DNA synthesome, an in vitro DNA replication model system that is fully competent to carry out all phases of the DNA replication process mediated by human cells (Dai et al., 2009). This study found a reduction of the fidelity and an inhibition of DNA synthesis that led to a dose-dependent increase in mutation frequency following intracellular exposure to trivalent chromium. Thus, hexavalent chromium can lead to the disruption of DNA synthesis and gene transcription at multiple levels, corresponding to an observable, dose-dependent increase in mutation frequency in human cells.

Epigenetic effects have also been observed following hexavalent chromium exposure. Epigenetic modifications, defined as heritable changes in gene expression that occur without altering the genetic material (Sharma et al., 2010), can drive malignant cellular transformation. These modifications can effect methylation, phosphorylation, gene expression, and cell signaling (Holmes et al., 2008). Cellular signaling involved in cell survival may be affected; a study exposing human bronchial epithelial cells in vitro found that soluble hexavalent chromium could inhibit apoptosis via NF-kB activation and inhibition of p53 (Wang et al., 2004). DNA repair has also been shown to be sensitive to epigenetic modifications. In a study finding microsatellite instability in hexavalent chromium-induced lung tumors of chromate-exposed workers (Hirose et al., 2002), increased DNA methylation was observed in the promoter region of the tumor suppressor gene p16 and the MMR gene hMLH1, indicating that chromium can induce epigenetic effects (Kondo et al., 2006; Takahashi et al., 2005). Gene transcription has also been shown to be affected by exposure to hexavalent chromium in vitro via epigenetic mechanisms. Sun et al. (2009) found alterations in the levels of histone methylation in human lung A549 cells exposed to hexavalent chromium, indicating the capability of these exposures to lead directly to changes in gene expression. This evidence suggests that epigenetic effects may contribute to the carcinogenicity of hexavalent chromium and its reduced valence states once inside the cell.

Reduction intermediates: pentavalent and tetravalent chromium. Depending on the reductant involved and the concentration of hexavalent chromium present, various amounts of the unstable intermediates pentavalent and tetravalent chromium can be generated prior to reduction to the final stable oxidative +3 state. At lower levels of hexavalent chromium

exposure, intracellular concentrations of these reductants are sufficient to complete the reduction of hexavalent chromium to its trivalent state. However, at higher hexavalent chromium exposures, these levels are depleted, resulting in a higher yield of pentavalent chromium from the one-electron reducing thiols, GSH, and cysteine, as well as tetravalent chromium from the two-electron donating ascorbate. While pentavalent and tetravalent chromium can be short-lived states of chromium within the cell, they are DNA reactive and can participate in redox reactions, forming free radical species that can also damage DNA (Stearns and Wetterhahn, 1994).

Redox cycling of the chromium ions can occur intracellularly when they are formed during reduction of hexavalent chromium. The process of hexavalent chromium reduction by GSH is accompanied by the reduction of molecular oxygen, yielding superoxide radicals. Reduction by GSH has been shown to involve the formation of GSH-derived thiyl radicals that can directly damage DNA or react with other thiols to also generate superoxide radicals. These radical species will react with hydrogen peroxide to produce hydroxyl radicals via Haber-Weiss reactions (Shi et al., 1999). Both hydrogen peroxide and superoxide radicals can participate in redox reactions involving both the pentavalent and tetravalent transition states of chromium that can generate hydroxyl radicals via Fenton and Haber-Weiss reactions (Shi et al., 1999). Hydroxyl radicals can directly react with genetic material, forming DNA-protein crosslinks, and DNA adducts with proteins and amino acids, damaging DNA bases, and producing DNA single- and double-strand breaks (reviewed in Kasprzak, 1996).

Although less frequent than the low molecular weight nonenzymatic reductants, reduction of hexavalent chromium can also occur by NAD(P)H-dependent flavoenzymes, including GSH reductase, lipoyl dehydrogenase, and ferredoxin-NADP+ oxidoreductase (Shi and Dalal, 1990). These enzymes catalyze a one-electron reduction that can result in the formation of stable pentavalent chromium−NADPH complexes that can react with hydrogen peroxide to generate hydroxyl radicals (Shi et al., 1999). The ability to form complexes with biological ligands allows stabilization of pentavalent, but not tetravalent, chromium intermediates (Levina and Lay, 2005). These pentavalent chromium−NADPH complexes have been shown to form in vitro in *E. coli* (Shi et al., 1991) and in vivo in mice (Liu et al., 1995).

Two other important nonenzymatic reducers of hexavalent chromium are ascorbate and cysteine. Ascorbate and cysteine are present at lower concentrations intracellularly than GSH, but they have kinetically faster rates of hexavalent chromium reduction. Ascorbate has been shown to yield pentavalent and tetravalent chromium and radical species when the intracellular ratio of ascorbate to chromium is <3:1 (Stearns and Wetterhahn, 1994). The precise nature of the radical species relevant to DNA damage is not known, however, and the degree of damage attributable to oxidative mechanisms is the subject of much debate. One study found an increase in mutations and replication-blocking DNA lesions in human fibroblasts resulting from the ascorbate-driven reduction of hexavalent chromium, but concluded that the mechanism responsible did not involve oxidative radicals, in part because the DNA damage anticipated by

species including hydroxyl radicals and pentavalent chromium-peroxo complexes, namely abasic sites and strand breaks was not observed (Quievryn et al., 2003). This study also found that no mutagenesis occurred in the presence of a trivalent chromium chelator, indicating the involvement of trivalent chromium−DNA adducts (see previous section). Similarly, studies of the DNA damage resulting from the intracellular reduction of hexavalent chromium by cysteine have shown that, while the intermediate species pentavalent and tetravalent chromium and thiyl radicals were formed, they were not responsible for DNA damage; rather, the trivalent chromium−DNA adducts were found to be the mutagenic species (Zhitkovich et al., 2001). The same group also found an elimination of mutagenicity when GSH reduction of hexavalent chromium occurred in the presence of phosphate ions that led to the sequestration of trivalent chromium, preventing its binding to DNA (Guttmann et al., 2008).

The ability of these intermediate chromium species to generate damaging free radicals is not in doubt, however, and there is evidence of reactive oxygen species generated by pentavalent chromium causing DNA damage. A decrease in DNA strand breaks was observed when hexavalent chromium reduction with GSH occurred in the presence of free radical scavengers (Kortenkamp et al., 1990). In addition, DNA double-strand breaks in subcellular systems were observed when ascorbate-mediated reduction of hexavalent chromium generated hydroxyl radicals via a Fenton-like reaction (Shi et al., 1994).

In an attempt to explain these conflicting results, it has been theorized that the responsible free radicals may be chromium-based and not oxygen-based radicals. This is due to the observation that the mutational spectra observed by chromium-induced radicals differs from that expected by damage due to reactive oxygen species that are generated following exposure to hydrogen peroxide, x-rays, or ionizing radiation (Sugden and Stearns, 2000). Hexavalent chromium has been shown to induce the formation of 8-oxo-deoxyguanosine adducts that are known to be induced by oxidative damage (Sander et al., 2005), but these lesions have also been shown to be induced directly by pentavalent chromium, with the subsequent addition of molecular oxygen (Sugden and Martin, 2002). In addition, the oxidant-sensitive dyes used to detect reactive oxygen species intracellularly can also be oxidized directly by pentavalent chromium and chromium-based radicals (O'Brien et al., 2003). Therefore, the induction of mutagenic lesions by the intracellular reduction of hexavalent chromium could be attributed to nonoxygen-dependent mechanisms.

Pentavalent chromium has been detected using EPR spectroscopy following intraperitoneal administration of hexavalent chromium in vivo, both in the liver and RBCs of chick embryos (Liebross and Wetterhahn, 1992), and in mouse liver and blood (Liu et al., 1994). In vitro, levels of DNA strand breaks were found to correlate with increasing levels of pentavalent chromium in Chinese hamster V79 cells (Sugiyama et al., 1989). Another in vitro study in human leukemic T-lymphocyte MOLT4 cells detected pentavalent chromium species and hydroxyl radicals with EPR following exposure to hexavalent chromium (Mattagajasingh et

al., 2008). The same study also observed a dose-dependent increase in protein carbonyls and malondialdehyde generated via protein oxidation and lipid peroxidation, respectively, although the lipid peroxidation only occurred significantly at much higher exposures of chromate (≥100 μM) compared with the protein oxidation, which was significant as low as 10 μM. Tetravalent chromium has been more difficult to observe due to its unstable nature compared to pentavalent chromium, but this species was shown to induce mitotic recombination in the somatic wing spot assay in Drosophila (Katz et al., 2001). Both species caused an induction of NF-kB, a nuclear transcription factor involved in the cellular response to oxidative damage, in cultured Jurkat cells. This activation was enhanced by hydrogen peroxide and eliminated when catalase was added to decompose hydrogen peroxide, indicating that hydroxyl radicals may have had a role (Shi et al., 1999).

In summary, there are many potential mechanisms involved in the genotoxicity of hexavalent chromium as a result of intracellular reduction. Intermediate valence states can react directly and indirectly through coordinate complexes with DNA as well as form radical species, and the final reduction product, trivalent chromium, can form various damaging DNA adducts. Additionally, significant evidence points to the aberrant processing of DNA mismatches induced by chromium−DNA adducts, leading to apoptosis of the damaged cells, or further promotion of these mutagenic lesions as the DNA double-strand breaks generated are substrates for error-prone repair processes such as nonhomologous end joining.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS—ORAL

In humans, several case reports have been published on clinical signs and symptoms in individuals following acute accidental or intentional ingestion of high doses (fatal or near fatal) of hexavalent chromium compounds, including chromic acid, potassium dichromate, and ammonium dichromate. Clinical presentation of patients following acute, high-dose exposure was similar, regardless of the specific hexavalent chromium compound ingested, and included the following: abdominal pain, nausea, and vomiting; hematemesis and bloody diarrhea; caustic burns of mouth, pharynx, esophagus, stomach, and duodenum and GI hemorrhage; anemia, decreased blood Hgb, abnormal erythrocytes, and intravascular hemolysis; hepatotoxicity (hepatomegaly, jaundice, elevated blood bilirubin, and liver enzyme activities); renal failure (oliguria and anuria); cyanosis; and metabolic acidosis, hypotension, and shock. Findings on tissue biopsies included hepatic fatty degeneration and necrosis and renal tubular degeneration and necrosis.

Information on chronic human health effects resulting from exposure to hexavalent chromium comes from several studies of human populations unknowingly consuming food or drinking water contaminated with hexavalent chromium over some extended time period. These studies have been primarily focused on cancer. However, the noncancer effects that have been recorded are consistent with the GI effects observed following acute exposures to hexavalent

chromium and have included oral ulcers, diarrhea, abdominal pain, dyspepsia, stomach pain, and vomiting (JinZhou Antiepidemic Station, 1979).

Table 4-26 presents a summary of studies of the noncancer effects of hexavalent chromium exposure from repeat-dose oral toxicity studies in experimental animals. The most sensitive targets of toxicity identified in these studies included the blood, liver, and GI tract. The effects seen in these target organs are more specifically discussed below.

Table 4-26. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to hexavalent chromium

Species	Sex	Exposure level[a]	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the NOAEL/LOAEL	Reference
Subchronic studies							
F344/N rat	F, M	0, 1.7, 3.5, 5.9, 11.2, or 20.9 mg/kg-d via drinking water	3 mo	F: ND M: ND	1.7 1.7	F: Microcytic, hypochromic anemia (decreased Hgb, MCV, MCH), increased serum liver enzyme activities (ALT and SDH) and bile acids, and histopathological changes to the duodenum (histiocytic cellular infiltration). M: Microcytic, hypochromic anemia (decreased Hct, Hgb, MCV, MCH), increased serum liver enzyme activities (ALT and SDH), and histopathological changes to pancreatic lymph nodes (histiocytic cellular infiltration).	NTP, 2007
$B6C3F_1$ mouse	F, M	0, 3.1, 5.3, 9.1, 15.7, or 27.9 mg/kg-d via drinking water	3 mo	F: ND M: ND	3.1 3.1	Histopathological changes (histiocytic cellular infiltration) in the duodenum.	NTP, 2007
$B6C3F_1$, BALB/c, and *am3*-C57BL/6 mouse	M	0, 2.8, 5.2, or 8.7 mg/kg-d via drinking water	3 mo	ND	2.8	Histopathological changes in the duodenum in $B6C3F_1$ mice (histiocytic cellular infiltration and epithelial hyperplasia), BALB/c mice (histiocytic cellular infiltration), and *am3*-C57BL/6 mice (epithelial hyperplasia).	NTP, 2007
Wistar rat	M	0 or 73.05 mg/kg-d via drinking water	30 d	ND	ND	Decreased serum prolactin levels. Data not adequate for estimation of a NOAEL or LOAEL.	Quinteros et al., 2007
Wistar rat	M	0 or 20 mg/L in drinking water	10 wks	ND	ND	Liver histopathologic changes. Doses in mg hexavalent chromium/kg-d could not be estimated.	Rafael et al., 2007
Wistar rat	M	0 or 1.5 mg/kg-d via drinking water	22 wks	ND	1.5	Changes in serum enzymes; liver triglycerides, glycogen and cholesterol; liver histopathologic changes.	Acharya et al., 2001
Swiss mouse	M	0, 177, 265, 353, 530, or 706 mg/L in drinking water	8 wks	ND	ND	Liver histopathologic changes. Doses in mg hexavalent chromium/kg-d could not be estimated.	Asmatullah and Noreen, 1999

Table 4-26. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to hexavalent chromium

Species	Sex	Exposure level[a]	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the NOAEL/LOAEL	Reference
Wistar rat	F	0 or 1.4 mg/kg-d via drinking water	22 wks	ND	1.4	Changes in liver weight; serum enzyme levels, triglycerides, glucose; liver glycogen; liver histopathology.	Chopra et al., 1996
Wistar rat	F, M	F: 0 or 1.76–2.47 mg/kg-d via drinking water M: 0 or 1.4–2.18 mg/kg-d via drinking water	6 mo	ND	ND	Changes in urinary markers of renal function. No histopathologic examination of the kidney.	Vyskocil et al., 1993
Chronic studies							
F344/N rat	F, M	F: 0.24, 0.94, 2.4, or 7.0 mg/kg-d via drinking water M: 0.21, 0.77, 2.1, or 5.9 mg/kg-d via drinking water	2 yrs	F: ND M: 0.21	0.24 0.77	F: Increased incidence of chronic inflammation of the liver. M: Increased incidences of nonneoplastic histopathological changes to the liver (basophilic foci), duodenum (histiocytic cellular infiltrate), and mesenteric lymph nodes (histiocytic cellular infiltrate and hemorrhage).	NTP, 2008
B6C3F$_1$ mouse	F, M	F: 0.38, 1.4, 3.1, or 8.7 mg/kg-d via drinking water M: 0.38, 0.91, 2.4, or 5.9 mg/kg-d via drinking water	2 yrs	F: ND M: ND	0.38 0.38	F: Increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia), mesenteric lymph nodes (histiocytic cellular infiltration), liver (histiocytic cellular infiltration), and pancreas (depletion of cytoplasmic zymogen granules). M: Increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia) and mesenteric lymph nodes (histiocytic cellular infiltration).	NTP, 2008
Dog	Not specified	0, 0.45, 2.25, 4.5, 6.75, or 11.2 mg/L in drinking water	4 yrs	ND	ND	No effects were observed. Doses in mg hexavalent chromium/kg-d could not be estimated.	Anwar et al., 1961

Table 4-26. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to hexavalent chromium

Species	Sex	Exposure level[a]	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the NOAEL/LOAEL	Reference
Sprague-Dawley rat	F, M	0.05–2.8 mg/kg-d via drinking water	1 yr	2.4–2.8	ND	No adverse effects observed at the highest dose tested.	MacKenzie et al., 1958
Reproductive/developmental studies							
Bonnet monkey	M	0, 1.0, 2.1, 4.1, and 8.3 mg/kg-d via drinking water	180 d	ND	2.1	Reversible changes to male reproductive organs, including disruption of spermatogenesis, effects on sperm count and velocity, and histopathological changes.	Aruldhas et al., 2006, 2005, 2004; Subramanian et al., 2006
Charles Foster rat	M	0, 20, 40, or 60 mg/kg-d via gavage	90 d	ND	20	Decreased serum testosterone levels and loss of 3β-Δ5-HSH activity in testes.	Chowdhury and Mitra, 1995
Wistar rat	M	0, 5.2, or 10.4 mg/kg-d via gavage	6 d	ND	5.2	Decreased sperm counts and histopathological changes to the testes.	Li et al., 2001
BALB/c mouse	M	0, 6.4, 12.7, or 25.5 mg/kg-d via gavage	35 d	ND	6.4	Increased percentage of degenerated tubules, undergenerated tubules without spermatogonia, abnormal sperm, and reduced number of spermatogonia.	Zahid et al., 1990
New Zealand White rabbit	M	0 or 3.6 mg/kg-d via gavage	10 wks	ND	3.6	Decreased testes and epididymis weight and decreased sperm output.	Yousef et al., 2006
Sprague-Dawley rat	F, M	F: 0, 0.25, 1.1, 2.5, or 9.9 mg/kg-d via the diet M: 0, 0.35, 1.1, 2.1, or 8.5 mg/kg-d via the diet	9 wks	F: 2.5 M: 2.1	F: 9.9 M: 8.5	F: Slight erythrocyte microcytosis. M: Slight erythrocyte microcytosis.	NTP, 1996b

Table 4-26. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to hexavalent chromium

Species	Sex	Exposure level[a]	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the NOAEL/LOAEL	Reference
BALB/c mouse	F, M	F: 0, 1.8, 5.6, or 12.0, 48.4 mg/kg-d via the diet M: 0, 1.1, 3.5, 7.4, or 32.5 mg/kg-d via the diet	9 wks	F: 1.8 M: 3.5	F: 5.6 M: 7.4	F: Histopathological changes to the liver (cytoplasmic vacuolization). M: Histopathological changes to the liver (cytoplasmic vacuolization).	NTP, 1996a
BALB/c mouse	F	0, 7.9, 16.1, or 37.1 mg/kg-d via the diet (F1 generation)	Continuous breeding study	ND	7.9	Erythrocyte microcytosis (slight decrease in MCH) in the F1 generation.	NTP, 1997
Druckrey rat	F	0, 70, 127, or 170 mg/kg-d via drinking water	3 mo	ND	70	Dam: Increased pre- and postimplantation losses. Offspring: Decreased fetal weight and external and skeletal abnormalities.	Kanojia et al., 1998
Swiss mouse	F	0, 63, 119, or 174 mg/kg-d via drinking water	GDs 6 through 14	ND	63	Dam: Decreased fertility. Offspring: Decreased fetal body weight and delays in skeletal development.	Junaid et al., 1996a
Wistar rat	F	0 or 7.9 mg/kg-d via drinking water	GDs 6 through 15	ND	7.9	Dam: Increased preimplantation loss/litter, postimplantation loss/litter, resorptions/litter, and dead fetuses/litter and decreased live fetuses/litter. Offspring: Decreased fetal weight and increased litters with fetal abnormalities or malformations including visceral and skeletal changes.	Elsaieed and Nada, 2002
Sprague-Dawley rat	F	0 or 35 mg/kg-d via drinking water	GDs 1–3 or 4–6	ND	35	Dam: Impaired implantation, increased resorptions, and decreased number of viable fetuses.	Bataineh et al., 2007
ITRC-Bred mouse	F	0, 48, 98, or 239 mg/kg-d via drinking water	Entire gestational period	Dam: 48 Offspring: ND	Dam: 98 Offspring: 48	Dam: Decreased body weight gain and increased resorptions and postimplantation loss. Offspring: Decreased fetal length and weight.	Trivedi et al., 1989

Table 4-26. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to hexavalent chromium

Species	Sex	Exposure level[a]	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the NOAEL/LOAEL	Reference
Swiss mouse	F	0, 53, 101, or 152 mg/kg-d via drinking water	20 days prior to mating	Dam: 53 Offspring: ND	Dam: 101 Offspring: 53	Dam: Decreased body weight gain and reduced number of implantation sites.	Junaid et al., 1996b
Swiss mouse	F	0, 53, 101, or 152 mg/kg-d via drinking water	GDs 14 through 19	Dam: 53 Offspring: ND	Dam: 101 Offspring: 53	Dam: Decreased body weight gain. Offspring: Reduced fetal weight and length and increased incidence of reduced caudal ossification.	Junaid et al., 1995

[a]Unless otherwise noted, dose or concentration expressed as hexavalent chromium.

F = female; M = male; ND = not determined

In regard to hematological effects, NTP (2007) observed microcytic, hypochromic anemia (i.e., decreased Hct, Hgb, MCV, and MCH) at a dose of 1.7 mg/kg-day of hexavalent chromium in both male and female F344/N rats in a 3-month (subchronic) study. In this same study, NTP (2007) also saw histopathological changes (i.e., histiocytic cellular infiltration) in the pancreatic lymph nodes in male F344/N rats at 1.7 mg/kg-day of hexavalent chromium. Finally, in a chronic (2-year) study, NTP (2008) observed histopathological changes (i.e., histiocytic cellular infiltration) in the mesenteric lymph nodes in male F344/N rats at 0.77 mg/kg-day of hexavalent chromium and male and female $B6C3F_1$ mice at 0.38 mg/kg-day of hexavalent chromium.

In the NTP (2007) subchronic study referenced above, liver effects were also observed at 1.7 mg/kg-day of hexavalent chromium and included increased serum liver enzyme activities (i.e., ALT and SDH) in both males and females and increased bile acids in females. In their 2-year bioassay, NTP (2008) found an increased incidence of chronic inflammation of the liver at 0.24 mg/kg-day of hexavalent chromium in female F344/N rats and increased incidences of histopathological changes to the liver (i.e., basophilic foci) at 0.77 mg/kg-day of hexavalent chromium in male F344/N rats. In this same bioassay, increased incidences of histopathological changes to the liver (i.e., histiocytic cellular infiltration) were seen at 0.38 mg/kg-day of hexavalent chromium in female $B6C3F_1$ mice.

Effects of hexavalent chromium ingestion on the GI tract have been primarily observed in the small intestine (duodenum). In a 3-month study, NTP (2007) saw histopathological changes to the duodenum in male F344/N rats at 1.7 mg/kg-day of hexavalent chromium, in male and female $B6C3F_1$ mice at 5.3 mg/kg-day of hexavalent chromium, and in male BALB/c and *am3*-C57BL/6 mice at 2.8 mg/kg-day of hexavalent chromium. These changes included diffuse epithelial hyperplasia and histiocytic cellular infiltration. In their 2-year study, NTP (2008) also found increased incidences of histopathological changes to the duodenum in male F344/N rats at 0.77 mg/kg-day of hexavalent chromium and in male and female $B6C3F_1$ mice at 0.38 mg/kg-day of hexavalent chromium. Similar to that observed in the subchronic study, these changes in the duodenum included diffuse epithelial hyperplasia and histiocytic cellular infiltration.

Animal studies also provide evidence that oral exposure to hexavalent chromium compounds produces reproductive effects, including histopathological changes to reproductive organs in males (Aruldhas et al., 2006, 2005, 2004; Li et al., 2001; Chowdhury and Mitra, 1995; Zahid et al., 1990) and females (Murthy et al., 1996); alterations in sperm, including decreased count, decreased motility, and abnormal morphology (Subramanian et al., 2006; Yousef et al., 2006; Li et al., 2001; Zahid et al., 1990); decreased plasma testosterone levels (Yousef et al., 2006; Chowdhury and Mitra, 1995); increased estrous cycle length (Kanojia et al., 1998, 1996; Murthy et al., 1996); changes in mating behavior and decreased fertility in males (Bataineh et al., 1997); and adverse reproductive outcomes, including decreased numbers of live fetuses and

implantations, and increased numbers of resorptions and pre- and postimplantation losses (Bataineh et al., 2007; Elsaieed and Nada, 2002; Kanojia et al., 1998, 1996; Elbetieha and Al-Hamood, 1997; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989). These reproductive toxicity studies are summarized in Table 4-26.

Developmental effects observed in animal studies have included decreased fetal weight and length (Elsaieed and Nada, 2002; Kanojia et al., 1998; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989); external (subdermal hemorrhage and tail malformations) and skeletal abnormalities (decreased ossification) (Elsaieed and Nada, 2002; Kanojia et al., 1998, 1996; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989); and delayed sexual maturation and function in female offspring (Banu et al., 2008; Al-Hamood et al., 1998). These effects were seen at hexavalent chromium doses ranging from about 2 to 100 mg/kg-day. These studies and the developmental effects observed are also summarized in Table 4-26.

In contrast to results of the above studies on reproductive toxicity, reproductive effects were not observed in dietary exposure studies conducted by NTP that investigated the potential effects of hexavalent chromium on male reproductive organs in rats and mice (NTP, 1996a,b) and on reproductive outcomes in a continuous breeding study in mice (NTP, 1997). The reason for the inconsistent results between the NTP studies and the other reproductive toxicity studies of hexavalent chromium are not readily apparent, as daily dose ranges evaluated in the NTP studies overlapped with those used in the other studies showing hexavalent chromium-induced reproductive effects.

Based on a review of the NOAELs and LOAELs in Table 4-26, the most sensitive hexavalent chromium-induced effects in rats were increased incidence of chronic inflammation of the liver in females and increased incidences of nonneoplastic histopathological changes to the liver (basophilic foci), duodenum (histiocytic cellular infiltrate), and mesenteric lymph nodes (histiocytic cellular infiltrate and hemorrhage) in males. In mice, the most sensitive hexavalent chromium-induced effects were increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia), mesenteric lymph nodes (histiocytic cellular infiltration), liver (histiocytic cellular infiltration), and pancreas (depletion of cytoplasmic zymogen granules) in females and increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia) and mesenteric lymph nodes (histiocytic cellular infiltration) in males. All of these effects were observed in the 2-year chronic study by NTP (2008), and in general, occurred at lower doses than the reproductive or developmental effects.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), hexavalent chromium is “likely to be carcinogenic to humans” via the oral route of exposure based on a statistically significant increase in the incidence of tumors of the oral mucosa and

tongue of rats and of the small intestine of mice; and evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans. Additionally, available evidence indicates that chromium interacts with DNA, resulting in DNA damage and mutagenesis. Thus, hexavalent chromium is proposed to induce carcinogenicity via a mutagenic mode of action.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

Human studies in which health outcomes (primarily cancer) were evaluated among populations that resided near sources of industrial waste containing hexavalent chromium compounds and unknowingly consumed hexavalent chromium in drinking water provide some evidence of possible associations between oral exposure to hexavalent chromium and cancer. These epidemiological studies evaluated populations in Liaoning Province, China (Kerger et al., 2009; Beaumont et al., 2008; Zhang and Li, 1997, 1987), Kings County/San Bernardino County, California (Fryzek et al., 2001), Nebraska (Bednar and Kies, 1991), and Glasgow, United Kingdom (Eizaguirre-Garcia et al., 2000, 1999) that unknowingly were exposed to hexavalent chromium over some time period. Of these studies, the most detailed analyses were of data collected from the JinZhou area of Liaoning Province, China, where groundwater, surface water, and agricultural soils were contaminated with chromium derived from hexavalent chromium production (e.g., 0.001–20 mg chromium/L in residential well water). This study found evidence of an excess risk of mortality from stomach cancer from 1970 to 1978 in residents of the area, relative to the reference populations (four other areas in Liaoning Province, and the total population of the province) (Beaumont et al., 2008). The association with stomach cancer mortality was weaker when an urban area was excluded from the reference population (Kerger et al., 2009). However, there was little difference between stomach cancer rates in urban compared to rural areas during this period, indicating no sound rationale for excluding this urban area from the reference group. Studies of chromium-exposed populations in California and Nebraska (Fryzek et al., 2001; Bednar and Kies, 1991) found no significant correlation between cancer mortality and drinking water concentration, and the study of the population in Glasgow (Eizaguirre-Garcia et al., 2000, 1999) found no correlation between leukemia risk and distance from a former chromium processing facility (where elevated soil concentrations for hexavalent chromium were measured). Interpretation of the findings from these three studies is limited by the analysis of all cancer mortality (rather than individual cancer types) in the case of the California and Nebraska studies and leukemia only in the case of the Glasgow study.

Evidence of carcinogenicity in animals was provided by the NTP (2008) bioassay conducted in rats and mice. In this study, exposure of F344/N rats to sodium dichromate dihydrate in drinking water for 2 years resulted in a statistically significant increase in the incidence of squamous epithelial papillomas and carcinomas of the oral mucosa and tongue (noted by NTP as rare when compared with historical controls) at the highest exposure level

(average daily doses of 5.9 and 7.0 mg hexavalent chromium/kg-day in males and females, respectively), but not at the three lower exposure levels. NTP (2008) also exposed $B6C3F_1$ mice to sodium dichromate dihydrate in drinking water for 2 years and reported statistically significant increases in the incidence of adenomas and carcinomas of the small intestine in males and females at doses ≥2.4 and ≥3.1 mg hexavalent chromium/kg-day, respectively.

As discussed in detail in Section 4.6.3, hexavalent chromium is proposed to induce carcinogenicity via a mutagenic mode of action. The key precursor events leading to mutagenicity have been identified in animals and these events are anticipated to occur in humans and progress to tumors.

The "likely to be carcinogenic to humans" descriptor is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "carcinogenic to humans". The database supports this descriptor for hexavalent chromium exposure via the oral route. On the other hand, available evidence to support the descriptor of "carcinogenic to humans" was also considered.

The "carcinogenic to humans" descriptor indicates strong evidence of human carcinogenicity, and can be characterized by different combinations of evidence. One line of evidence indicates this descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer (U.S. EPA, 2005a). This is not the case for exposure to hexavalent chromium via ingestion. A moderately elevated risk of stomach cancer mortality was seen in JinZhou (Liaoning Province, China), but this risk has not been established in other populations exposed to drinking water contaminated with hexavalent chromium. The epidemiologic data are not sufficient to establish a causal association between exposure to hexavalent chromium by ingestion and cancer.

A second line of evidence under which this descriptor may be appropriate involves a lesser weight of epidemiologic evidence that is strengthened by other information, including strong evidence of an association between human exposure and either cancer or the key events of the mode of action and extensive evidence of carcinogenicity in animals (U.S. EPA, 2005a). As discussed above, the epidemiologic evidence for the oral route of hexavalent chromium exposure is not considered strong. In addition, extensive evidence of the carcinogenicity of hexavalent chromium in animals via ingestion does not exist. Only one multiple-dose chronic oral carcinogenicity study of hexavalent chromium in animals is available (i.e., the 2-year bioassay in rodents conducted by NTP [2008]). Taken together, these considerations do not provide a basis for the characterization of hexavalent chromium as "carcinogenic to humans" via oral exposure. Therefore, U.S. EPA concluded that, based on the available information, the descriptor "likely to be carcinogenic to humans" is the most appropriate descriptor for the carcinogenic potential of hexavalent chromium via ingestion.

4.7.3. Mode-of-Action Information

4.7.3.1. *Hypothesized Mode of Action*

The hypothesized mode of action for carcinogenicity induced by hexavalent chromium is via mutagenesis. The hypothesis is that carcinogenicity can be induced directly by reduced forms of chromium interacting with DNA to form adducts and crosslinks that can lead to DNA breaks and mutations, and indirectly by free radical species generated during the reduction process that can also lead to DNA breakage and mutagenesis.

Trivalent chromium is the ultimate product of the intracellular reduction of hexavalent chromium. Trivalent chromium is capable of interacting directly with DNA, forming stable coordination complexes with nucleic acids and peptides (Salnikow and Zhitkovich, 2008). In particular, trivalent chromium is capable of forming ternary complexes with DNA and an intracellular reducer, such as ascorbate, GSH, or cysteine (Zhitkovich et al., 1996; Salnikow et al., 1992), as well as crosslinking DNA and proteins, and forming intrastrand DNA-DNA crosslinks (Zhitkovich, 2005; Voitkun et al., 1998). These chromium-DNA complexes, as well as DNA-protein and DNA-DNA crosslinks, have the capability of causing DNA single- and double-strand breaks, which, if not adequately repaired, can lead to cell death, or if misrepaired, can result in mutation.

Thus, once inside the cell, hexavalent chromium, through reduction to its pentavalent, tetravalent, and trivalent forms, is capable of inducing a wide range of mutagenic and genotoxic damage, including the formation of DNA adducts, DNA-protein and DNA-DNA crosslinks, mutations, DNA single- and double-strand breaks, abasic sites, oxidized DNA bases, chromosomal aberrations, sister chromatid exchanges, and micronuclei.

Key events.

1. *Cellular uptake of hexavalent chromium.* The first key event in hexavalent chromium-induced carcinogenesis is cellular uptake of hexavalent chromium. Hexavalent chromium readily enters cells via nonspecific sulfate and phosphate transporters, which occurs due to the structural similarity of hexavalent chromium to these tetrahedral anions (Bridges and Zalups, 2005). If hexavalent chromium is reduced *before entering the cell*, very little chromium will be taken up, as cells are relatively impermeable to trivalent chromium (Standeven and Wetterhahn, 1989).
2. *Intracellular reduction of hexavalent chromium.* Once inside the cell, hexavalent chromium quickly undergoes a series of reduction reactions to yield pentavalent, tetravalent, and ultimately the thermodynamically stable trivalent chromium. These reactions are enabled by abundant nonenzymatic reductants within the cell, primarily GSH, ascorbate, and cysteine (reviewed in McCarroll et al., 2009).
3. *DNA damage via reduced chromium species.* Following this intracellular reduction, several possible mechanisms leading to mutagenicity can occur, since the products of hexavalent chromium reduction within the cell (pentavalent, tetravalent, and trivalent

chromium) have all been shown to be DNA reactive (O'Brien et al., 2003). Hexavalent chromium is reduced by GSH to yield pentavalent chromium and thiyl radicals, which can react with other thiol molecules to produce superoxide radicals. Both pentavalent and tetravalent chromium can participate in Fenton reactions, generating hydroxyl radicals (Salnikow and Zhitkovich, 2008; Volko et al., 2006). Hence, the next key event is the direct interaction of the reduced forms of chromium with DNA, leading to DNA single- and double-strand breaks, base modifications, lipid peroxidation, and overall genomic instability, which can lead to mutations if not adequately repaired.

4. *Apoptosis and clonal expansion of mutated cells.* Apoptosis induced by hexavalent chromium exposure is initiated by several pathways, both genotoxic and nongenotoxic, to eliminate the damaged cells from the population. In the process, cells that are resistant to apoptosis (due either to mutations caused by hexavalent chromium or pre-existing mutations) are selected for, allowing clonal expansion of cells that are capable of evading apoptosis.

4.7.3.2. *Experimental Support for the Hypothesized Mode of Action*

Strength, consistency, and specificity of association. A large database of experimental data exists on the mutagenic activity of hexavalent chromium compounds (these results are summarized in Section 4.4.1 and in the corresponding tables). In vitro, positive results were found in the majority of tests performed on hexavalent chromium compounds in bacterial test systems (see Table 4-21). Similarly, in yeast (*S. cerevisiae* and *S. pombe*), all available studies described positive results for the detection of gene mutations, mitotic gene conversion, and mitotic crossing over.

In mammalian cell lines and primary cells, all studies using whole cells in vitro yielded positive results (Table 4-22). Evidence of mutation induction was shown at the tk locus in the mouse lymphoma assay, as well as at the HGPRT locus in Chinese hamster ovary cells (V79 and AT3-2). In human cells, chromosome aberrations, DNA damage, and DNA-DNA and DNA-protein crosslinks were detected in primary cultures and established cell lines originating from target organs, including the gastric mucosa, bronchial epithelium, and fibroblasts from the bronchial tubes and lung. Chromosome aberrations, sister chromatid exchanges, and DNA damage were observed in primary human dermal fibroblasts and lymphocytes as well as bronchial fibroblasts and epithelial cells. Chromosome aberrations and DNA damage were found in mouse carcinogenic cell lines, and sister chromatid exchanges were detected in mouse blastocysts. In rats, DNA damage and unscheduled DNA synthesis were observed in rat gastric mucosal cells and hepatocytes as well as in primary lymphocytes, and transformation was observed in rat liver epithelial cells upon exposure to hexavalent chromium. A number of studies have been performed using cultured Chinese hamster ovary cells, showing chromosomal aberrations and sister chromatid exchanges as well as DNA damage, DNA-protein crosslinks,

and induced DNA methylation, and three studies showed induced transformation in cultured Syrian hamster embryo cells.

In vivo, most studies of the mutagenicity of hexavalent chromium compounds have yielded positive results (Table 4-23). Somatic and germ cell mutations were detected in 3-day-old *D. melanogaster* larvae fed potassium chromate, potassium dichromate, or calcium chromate (Kaya et al., 2002; Spano et al., 2001; Amrani et al., 1999; Graf and Wurgler, 1996; Zimmering et al., 1985). A number of in vivo oral exposure studies of the mutagenicity of hexavalent chromium in mice and rats are available, with slightly differing results depending on the method used. In the two studies in rats, Coogan et al. (1991) found DNA-protein crosslinks in liver and not in splenic lymphocytes following 3- or 6-week exposures of 100 or 200 mg/L in drinking water, but Mirsalis et al. (1996) did not find any evidence of DNA repair via unscheduled DNA synthesis in rat hepatocytes following 48-hour exposures of up to 20 mg/L in drinking water or a single gavage dose of 20 mL/kg at the same concentration. In other studies of mice exposed via gavage, DNA damage, as measured by the comet assay, was found in peripheral leukocytes (including isolated lymphocytes), stomach, colon, liver, kidney, bladder, lung, and brain (Wang et al., 2006; Devi et al., 2001; Sekihashi et al., 2001), but neither DNA damage nor micronuclei were found in bone marrow (De Flora et al., 2006; Sekihashi et al., 2001; Shindo et al., 1989). Similarly, in studies of mice exposed via drinking water, De Flora et al. (2008, 2006) reported negative results for the detection of micronuclei in the bone marrow of pregnant Swiss albino mice and in the fetal polychromatic erythrocytes after exposures up to 20 mg/L and also in adult BDF_1 mice following 500 mg/L exposure for 210 days.

Interestingly, NTP (2007) investigated micronuclei induction in male mouse bone marrow following a 3-month drinking water exposure and found differing results depending on the strain of mouse used. In one phase of the study, results were negative in $B6C3F_1$ mice exposed to doses as high as 349 mg/L, while in another phase, following exposures of 0, 21.8, 43.6, or 87.2 mg/L hexavalent chromium, results were negative in BALB/c mice, equivocal in $B6C3F_1$ mice, and significantly positive at ≥43.6 mg/L exposures in *am3*-C57BL/6 mice, with a statistically significant positive trend starting at 21.8 mg/L.

Somatic and germ cell mutations were detected in *D. melanogaster* treated intraperitoneally with chromic acid or potassium dichromate (Rodriguez-Arnaiz and Martinez, 1986) or with sodium dichromate via filter paper (Rasmuson, 1985). Following parenteral exposure in mice, DNA damage was detected in the stomach, colon, bladder, lung, brain, liver, and kidney (Sekihashi et al., 2001; Ueno et al., 2001; Amlacher and Rudolph, 1981); mutations were found in the liver of transgenic mice (Itoh and Shimada, 1998, 1997), in the germ cells of hybrid male mice (Paschin et al., 1982), and in the offspring of exposed female mice (Knudsen, 1980); and micronuclei were increased in bone marrow and polychromatic erythrocytes (De Flora et al., 2006; Wronska-Nofer et al., 1999; Itoh and Shimada, 1996; Hayashi et al., 1982; Paschin and Toropzev, 1982; Wild, 1978), as well as in the liver and peripheral blood of mice

exposed prenatally (De Flora et al., 2006). In rats exposed parenterally, DNA damage was detected in leukocytes (Patlolla and Tchounwou, 2006), and DNA-protein crosslinks were found in lung, liver, and kidney (Tsapakos et al., 1983). Mutations were observed in the lung and kidney from transgenic mice exposed intratracheally to hexavalent chromium (Cheng et al., 2000); DNA-protein crosslinks and DNA fragmentation and adducts were found in the lung of rats similarly exposed (Izzotti et al., 1998), while in rats exposed via inhalation, chromosomal aberrations and sister chromatid exchanges were observed in peripheral lymphocytes (Koshi et al., 1987).

In addition to the in vivo evidence in animals for the genotoxicity of hexavalent chromium, several studies are available in humans (Table 4-24). In the only mutagenicity study following oral doses, DNA-protein crosslinks were not detected in peripheral lymphocytes up to 4 hours after the four volunteers were given 71 µg hexavalent chromium/kg (Kuykendall et al., 1996). Another study (Gao et al., 1994) failed to detect DNA damage in peripheral lymphocytes of workers inhalationally exposed to 0.001–0.055 mg/m^3. However, several studies of occupational exposures via inhalation provide evidence of significant levels of chromium-induced DNA damage (Gambelunghe et al., 2003), and the formation of micronuclei (Benova et al., 2002; Vaglenov et al., 1999), chromosomal aberrations (Deng et al., 1988; Sarto et al., 1982), and sister chromatid exchanges (Wu et al., 2001, 2000; Deng et al., 1988; Sarto et al., 1982; Stella et al., 1982) in peripheral lymphocytes and/or buccal mucosal cells. These studies detected genotoxicity in workers exposed to mean air concentrations as low as 0.0075 and 0.0249 mg/m^3 (Benova et al., 2002). In addition, three studies found negative results for micronuclei and sister chromatid exchange, but the exposure concentrations were not reported (Nagaya et al., 1991; Sarto et al., 1990; Nagaya, 1986).

Dose-response concordance and temporal relationship. As noted above, hexavalent chromium is hypothesized to induce carcinogenicity via a mutagenic mode of action. The initial key events in the hypothesized mutagenic mode of action are the capability of the hexavalent form of chromium to pass through the cell membrane and, once inside, to be reduced to pentavalent, tetravalent, and trivalent chromium.

The available animal studies show that hexavalent chromium induces tumors in the tongue, oral mucosa, and intestines of rodents (NTP, 2008). Studies of a human cohort in Liaoning Province, China, exposed to 0.001–20 mg chromium/L in residential well water (Beaumont et al., 2008; Zhang and Li, 1997, 1987) reported an excess risk of mortality from stomach cancer in residents of the area. However, this risk has not been established in other human populations exposed to drinking water contaminated with hexavalent chromium, and thus the epidemiologic data are not sufficient to establish a casual association between exposure to hexavalent chromium by ingestion and cancer.

NTP (2008) reported a statistically significant increase in the incidence of tumors of the oral mucosa and tongue in rats exposed to hexavalent chromium for 2 years in drinking water at

average daily doses of 5.9 and 7.0 mg/kg-day for males and females, respectively, and tumors of the small intestine in mice exposed to average daily doses of ≥2.4 and 3.1 mg/kg-day in males and females, respectively. Correlating these data with mutagenicity testing by establishing temporal and dose and/or site concordance can be difficult, as in vivo assays designed to detect mutagenicity are conducted within a relatively short time after the exposure period has ended, and tend to rely mainly on cells from tissues such as bone marrow and/or blood that are actively replicating and therefore sensitive to mutagenic agents. There is evidence, however, that hexavalent chromium can accumulate and induce mutagenicity in tissues at the site of entry and systemically, at doses relevant to human exposures.

Following drinking water exposures, only one animal study has directly investigated target tissue genotoxicity (De Flora et al., 2008). With regard to dose, the De Flora et al. (2008) study tested levels (5 and 20 mg/L, or 1.2 and 4.82 mg/kg-day of hexavalent chromium) that were just below those leading to murine intestinal (duodenum, jejunum, and ileum) tumors in the 2-year NTP study (30 and 50 mg/L for males and females, respectively). Negative results were reported for DNA-protein crosslinks and DNA adducts when measuring the forestomach, glandular stomach, and duodenum of mice exposed to hexavalent chromium for 9 months via drinking water. However, the shorter study duration of DeFlora et al. (2008) makes a direct comparison of these results to the duodenal tumors reported in the chronic NTP bioassay infeasible.

Other studies have shown evidence of in vivo genotoxicity in nontarget tissues at early time points following exposure. In three studies that used the comet assay to detect DNA damage following gavage exposures in mice, Devi et al. (2001) found evidence of DNA damage in leukocytes that peaked at 48 hours postexposure, Wang et al. (2006) detected DNA damage in lymphocytes after 1- or 5-day consecutive exposures, and Seikihashi et al. (2001) detected DNA damage in stomach, colon, liver, kidney, bladder, lung, and brain within 8 hours of dosing that subsided by 24 hours posttreatment.

Devi et al. (2001) found positive dose-dependent results at >10-fold lower doses (0.21, 0.42, 0.84, 1.68, and 3.37 mg hexavalent chromium/kg). In fact, many of the positive in vivo mutagenicity studies found a positive trend with dose, including oral exposures (Wang et al., 2006; Devi et al., 2001) and parenteral exposures (Itoh and Shimada, 1996; Shindo et al., 1989; Hayashi et al., 1982; Paschin and Toropzev, 1982; Knudsen, 1980; Wild, 1978) in rats (Patlolla et al., 2008) and mice.

Therefore, the detection of DNA damage, a key event for the mutagenic mode of action following oral exposure to hexavalent chromium, which exhibits dose-dependence and is observed at time points prior to tumor development, strengthens the causal nature of this association. Although DNA-protein crosslinks and DNA adducts were not detected in target tissues following drinking water exposure in mice (De Flora et al., 2008), the lack of these

findings did not preclude the observation of mutations in other tissues and organs, considered to be early events following hexavalent chromium exposure leading to carcinogenesis.

Biological plausibility and coherence. Mutagenicity as a mode of action for carcinogenicity in humans is a biologically plausible mechanism for tumor induction. Hexavalent chromium has been shown to be mutagenic in vitro and in vivo, across species and tissue types. Human studies have shown induction of DNA damage, chromosomal aberrations, and micronucleus induction following exposure to hexavalent chromium, and in vivo animal studies show that hexavalent chromium induces DNA damage in rat blood, bone marrow, lung, liver, and kidney, and in mouse blood, lung, liver, kidney, bladder, colon, and brain. Exposures that induced a mutagenic response in these studies included doses within the range causing tumors in rats and mice in a chronic exposure bioassay (NTP, 2008).

Only one study examined tumor target tissue for evidence of mutagenicity (De Flora et al., 2008). De Flora et al. (2008) found negative results for DNA-protein crosslinks and DNA adducts in the duodenum in mice following drinking water exposures. Other available drinking water exposure studies of hexavalent chromium that measured mutagenicity in mice failed to show evidence of micronucleus induction in the blood or bone marrow (De Flora et al., 2008, 2006; NTP, 2007; Mirsalis et al., 1996).

It has been postulated (De Flora et al., 2008) that the positive results for DNA damage found in mice following gavage exposures (Wang et al., 2006; Devi et al., 2001; Sekihashi et al., 2001) were the result of overwhelming the reductive capacity of the GI tract in mice, allowing the accumulation and subsequent absorption of hexavalent chromium. This would indicate that the comparatively lower concentrations of hexavalent chromium administered in the drinking water studies (De Flora et al., 2008, 2006) are effectively reduced to trivalent chromium when ingested, thereby inhibiting cellular uptake and subsequent DNA damage. While this is a plausible explanation for the results following drinking water exposures, which are unusual in that they represent the only component of the hexavalent chromium mutagenicity database that does not show overwhelmingly positive results, there are inconsistencies with this explanation. For example, although the doses administered in De Flora et al. (2008) were lower than those in Wang et al. (2006) and Sekihashi et al. (2001), Devi et al. (2001) found positive results at doses approximately sixfold lower than the lowest dose used by De Flora et al. (2008).

In addition, genetic differences have been implicated in predicting the severity of genotoxic responses to hexavalent chromium exposure. In the 3-month NTP bioassay (2007), three different strains of mice ($B6C3F_1$, BALB/c, and *am3*-C57BL/6) were exposed to hexavalent chromium in drinking water at concentrations of 21.8, 43.6, or 87.2 mg/L, and different results for micronucleus induction in polychromatic erythrocytes were found among strains. The BALB/c mice showed no micronucleus induction, and results in the $B6C3F_1$ mice were equivocal at the highest dose of 87.2 mg/L and in the trend test ($p = 0.031$). However, the *am3*-C57BL/6 mice responded with a statistically significant overall positive trend, with the two

highest doses statistically significant, and the lowest dose nearly so. Based on the expected reduction capacity of an average 50 g mouse, it does not appear that the reductive capacities were overwhelmed in the NTP bioassay. The average rate of hexavalent chromium exposure for all three strains of mice was estimated to have been 2.9×10^{-2} mg/hour at the highest dose (NTP, 2007). This rate is within the estimated reductive capacity of the mouse GI tract of 4.4×10^{-2} mg/hour that is based on an estimated 0.33 mL/hour rate of drinking water consumption. However, the micronucleus results could reflect minor differences in the capacities of these three strains of mice to reduce hexavalent chromium extracellularly, since the exact reductive capacity of each mouse strain used is unknown.

The repair mechanisms in place for the resolution of DNA damage also appear to play a role in the carcinogenicity of hexavalent chromium. While ER has been shown to prevent hexavalent chromium-induced DNA damage (O'Brien et al., 2005), it has also been shown to be responsible for the generation of DNA damage following exposures (Brooks et al., 2008). Another DNA repair pathway important in resolving mismatched bases during DNA replication, MMR, has recently been implicated in the genotoxic responses to hexavalent chromium exposure. It has been shown that the processing of chromium-DNA adducts by the MMR pathway is responsible for turning these lesions into frank DNA double-strand breaks (Peterson-Roth et al., 2005). This group found that cells deficient in MMR were not subject to the same toxic responses to hexavalent chromium as were cells with these repair processes intact. This loss of MMR function leads to an unstable mutator phenotype, in which replication errors, particularly those occurring in simple nucleotide repeat sequences known as microsatellites, are not corrected, leading to an increase in mutation frequency (Loeb et al., 2008). Further, these effects would be exacerbated by the physical and chemical interference with DNA replication that occurs when trivalent chromium is present intracellularly (Eastmond et al., 2008).

There are several forms of cancer that exhibit microsatellite instability. For example, microsatellite instability has been implicated as the cause of the majority of cases of hereditary nonpolyposis colorectal cancer due to the inactivation of genes involved in the MMR pathway. In an epidemiological study of chromate-exposed workers, microsatellite instability was reported to occur in 79% of hexavalent chromium-induced lung tumors compared to only 15% in the nonchromate lung cancer group (Hirose et al., 2002). The same group also reported finding increased DNA methylation in the promoter region of the tumor suppressor gene p16 and the MMR gene hMLH1 in human lung cancers in these chromate-exposed workers, indicating that chromium can induce epigenetic effects (Kondo et al., 2006; Takahashi et al., 2005). These findings reflect a loss of functional MMR capability that could be mechanistically involved in chromate-induced lung cancer.

It was found that all four proteins responsible for MMR function were required for the processing of chromium-DNA adducts into DNA double-strand breaks (Peterson-Roth et al., 2005). The genes involved in MMR are known to be highly polymorphic in humans (Goode et

al., 2002), and given spontaneous background rates of mutation in human cells, it would not be unexpected to find small populations of cells that have acquired mutations in one of these four MMR genes. An inactivating mutation in any one of these would result in a growth advantage to cells exposed to hexavalent chromium, allowing them to evade apoptotic responses to these genotoxic lesions, as well as incurring further microsatellite instability, leading to a mutator phenotype. Thus, a selective advantage upon chronic exposure to even low levels of hexavalent chromium could translate into a clonal expansion of these MMR-deficient cells, leading to further evasion of cell death and increasing mutation frequencies, resulting in a state of genomic instability. This suggests that interindividual differences in the capacity and fidelity of DNA repair processes could determine susceptibility to ingested hexavalent chromium.

In summary, DNA damage can occur following oral exposure to hexavalent chromium at doses that should be within the reductive capacity of the organism. This DNA damage may be repaired by error-prone mechanisms, resulting in DNA double-strand breaks and microsatellite instability, and further exacerbated by both hexavalent chromium-induced epigenetic effects that alter these DNA repair mechanisms and the interference of DNA replication processes by hexavalent chromium. Genomic instability, or an increased rate of acquisition of genetic alterations, may result not only in the form of microsatellite instability but also as chromosomal instability and aneuploidy that have been shown to occur after prolonged exposure to hexavalent chromium. Exposure also results in complex alterations in gene expression that can alter cell survival pathways; this combined with apoptosis induced by both genotoxic and non-genotoxic mechanisms induced by hexavalent chromium can lead to a deregulation of cellular proliferation, resulting in the clonal expansion of cells that are resistant to apoptosis and eventually leading to neoplastic transformation.

In addition, it is of note that among the available oral exposure studies in mice, all studies that investigated DNA damage or micronucleus induction in bone marrow cells found negative results, including the study by Sekihashi et al. (2001), which found DNA damage in every tissue examined (liver, kidney, lung, brain, stomach, colon, and bladder) except for the bone marrow. The reason for the negative findings in these assays is unknown, but the high turnover of cells in the bone marrow may have allowed for more efficient repair of the damaged cells.

Bioavailability. As noted above, there is uncertainty surrounding the ability of hexavalent chromium to induce mutagenicity and carcinogenicity in humans considering the potential for reduced bioavailability. Intrinsic to the mutagenic and carcinogenic processes of hexavalent chromium is its ability to reach relevant tissues prior to being reduced to pentavalent, tetravalent, and trivalent chromium. When hexavalent chromium is reduced to the trivalent form extracellularly, this reduction process effectively detoxifies hexavalent chromium, since trivalent chromium is nearly impermeable to the cell.

Quantitative studies of GI absorption of hexavalent chromium in humans have estimated that as much as 10% of an ingested dose of 5 mg is absorbed (Kuykendall et al., 1996),

indicating that not all hexavalent chromium is reduced by the gastric juices of the stomach. In rats and mice, daily oral doses of 8 mg hexavalent chromium/day for 8 weeks resulted in absorption and accumulation of chromium in the bone, spleen, liver, and kidney (Kargacin et al., 1993); rats given 0.138 µmol hexavalent chromium/day for 3 days exhibited GI absorption of about 16% (Febel et al., 2001), and the absorption of 4–10% of a single daily dose of 57 µg hexavalent chromium (as $Na^{51}CrO_4$) was observed in rats, regardless of fasting state (MacKenzie et al., 1959). Distribution studies have shown that hexavalent chromium, once absorbed, distributes to nearly all tissues, particularly concentrating in the kidney, liver, bone, and RBCs. Thus, at oral doses within human exposure ranges, hexavalent chromium was not completely reduced by the GI tract, making available some portion of ingested hexavalent chromium to be absorbed directly by the mucosal cells of the GI tract, or to be distributed to other tissues throughout the body.

However, based on an understanding of chromium chemistry, as well as in vitro and in vivo studies conducted by De Flora et al. (2008, 1997), the reduction of at least some portion of ingested hexavalent chromium to trivalent chromium likely occurs in the GI tract (see Chapter 3). No data are currently available on the capacity of the rodent stomach to reduce hexavalent chromium. However, based on in vitro measurements, De Flora et al. (1997) estimated that the reductive capacity of the human GI tract is sufficiently large to effectively reduce even high doses of ingested hexavalent chromium to the less toxic trivalent form. Given this assertion, it is appropriate to ask whether the observed effects at the doses employed in the NTP (2008) study resulted from an exceedance of the reductive capacity of the rodent GI tract. This is important because if the effects observed only occurred due to the reductive capacity of the rodent GI tract being exceeded, these results may be less relevant to human risk at the lower doses that humans are more likely to be exposed.

In discussing the results of the NTP (2008) study, the original NTP investigators, Stout et al. (2009), specifically addressed this extracellular reduction issue. Qualitatively, Stout et al. (2009) noted that, in the 2-year NTP study, the observed increases in neoplasms of the small intestine of mice and the toxicity to the erythron, histiocytic infiltration, and uptake of hexavalent chromium into the tissues of rats and mice suggested that, under the conditions of this study, at least a portion of the administered hexavalent chromium was not reduced in the stomach. Moreover, Stout et al. (2009) also pointed out the significant disparity in the oral toxicity and carcinogenicity of hexavalent chromium versus trivalent chromium in rodents, including the absence of increases in neoplasms or nonneoplastic lesions of the small intestine in rats or mice exposed to chromium picolinate monohydrate, a trivalent chromium compound tested in an earlier NTP bioassay. Stout et al. (2009) believe that these data provide additional evidence that hexavalent chromium is not completely reduced in the stomach and is responsible for the observed effects.

In addressing the De Flora et al. (2008) suggestion that increases in neoplasms of the small intestine observed in mice are the result of a saturation of the gastric reduction capacity, Stout et al. (2009) took a more quantitative approach. Stout et al. (2009) postulated that if the threshold mechanism proposed by De Flora et al. (2008) actually existed, then the dose that saturated the reduction capacity would likely represent an inflection point on a sublinear dose-response curve, with doses above the inflection point demonstrating an increasing rate of response per unit dose. To test this hypothesis, Stout et al. (2009) evaluated tissue concentration and mouse small intestine neoplasm data for linearity and found that data that were statistically nonlinear were supralinear (i.e., exhibited a decreasing rate of response per unit dose), which does not support the presence of a reduction threshold.

Finally, De Flora et al. (1997) estimated the reductive capacity of human gastric juice to be about 84–88 mg of hexavalent chromium/day. Similar data are not available for the reductive capacity of mouse gastric juice. However, Stout et al. (2009) assumed that hexavalent chromium reduction is equally effective in mice and humans and that gastric secretion scales across species by body weight$^{3/4}$. Then, they estimated the reductive capacity of the gastric juice from a 50-g mouse to be approximately 0.4 mg/day (8 mg/kg-day). Stout et al. (2009) then pointed out that this value is greater than all of the male mouse doses and is nearly equivalent to the average daily dose of hexavalent chromium in the high-dose group of female mice in the NTP (2008) study. Therefore, Stout et al. (2009) concluded from their analysis that the neoplasms in the small intestine of mice occurred at dose levels that did not exceed the estimated hexavalent chromium reduction capacity of the gastric juices in mice.

4.7.3.3. *Other Possible Modes of Action*

In the carcinogenic process, aberrant cell survival, proliferation, and tissue remodeling are known contributors to the etiology of cancer (Hanahan and Weinberg, 2000). Evidence of diffuse duodenal hyperplasia in mice in all exposure groups was observed in the 3-month NTP (2007) study. The sites where hyperplasia was observed correlated with the site of tumors observed in the 2-year bioassay (NTP, 2008). One mechanism of cellular proliferation known to occur following exposures to xenobiotic agents involves that of toxicity causing cellular death and consequent regenerative cellular proliferation, comprising a potential mode of action for carcinogenesis. However, the study by NTP noted that no evidence of tissue damage or necrosis was observed in these animals, and most of the available studies of hexavalent chromium-induced genetic damage observed genotoxicity at doses below those inducing cytotoxicity, particularly in studies showing dose-dependent genetic damage.

Another acquired capability during tumor development is resistance to apoptosis, a hallmark of most if not all types of cancer (Hanahan and Weinberg, 2000). Apoptosis has been shown to occur following exposure to hexavalent chromium exposure (Flores and Perez, 1999; Ye et al., 1999; Singh et al., 1998). It is possible that the apoptotic cell death occurring after

hexavalent chromium exposure, initiated by ROS damage, altered cell signaling pathways, and genotoxic damage, occurs at levels that would not result in visible pathology or a regenerative response, but are significant enough to have an impact on the balance between cell survival and death. It has been proposed that in cells exposed to hexavalent chromium, apoptosis occurring in response to DNA damage, oxidative stress, or damage to mitochondria may serve as another key event in the carcinogenesis of hexavalent chromium, in that it allows for selection of cells that are resistant to apoptosis due to mutation and provides a means of clonal expansion for these cells (Nickens et al., 2010). DNA damage leads to the induction of cell cycle checkpoints to assess and repair the genetic damage; if the damage is too severe to repair, the cell will be targeted for cell death. This removes a potentially mutagenic cell from the population, but other cells deemed sufficient for repair could still exist and incur mutations following error-prone repair processes. In this manner, cells that have mutations enabling them to elude apoptosis, whether they were the result of hexavalent chromium mutagenesis or pre-existing, are conferred a growth advantage. Cells may also avoid targeted death due to changes in gene expression that lead to upregulation of pro-inflammatory and/or anti-apoptotic genes. These processes could be temporally similar to those of DNA damage and mutation, and may serve to lay the groundwork for the acquisition of other carcinogenic traits, including uncontrolled cell growth, leading to tumor formation. Therefore, rather than an alternate mode of action per se, apoptosis induced by hexavalent chromium exposure is considered here to be a key event in the carcinogenic process.

4.7.3.4. *Conclusions About the Hypothesized Mode of Action*

As noted above, hexavalent chromium is hypothesized to be carcinogenic by a mutagenic mode of action. The key events in the hypothesized mutagenic mode of action are the uptake of hexavalent chromium into the cell followed by intracellular reduction to pentavalent, tetravalent, and trivalent chromium. These reduced forms of hexavalent chromium and the free radicals that are formed during the reduction process are capable of directly interacting with cellular components, giving rise to mutagenicity (including DNA adduct formation, DNA damage, gene mutations, chromosomal aberrations, and micronuclei formation). Considering the database, there is evidence that hexavalent chromium can accumulate and induce mutagenicity in various tissues throughout the body at doses relevant to human exposures and, for oral exposures, within the reductive capacity of the GI tract.

(1) *Is the hypothesized mode of action sufficiently supported in the test animals?* The experimental evidence that hexavalent chromium is mutagenic, as presented in Section 4.4.1, includes multiple adverse genetic effects including DNA adduct formation, DNA damage, gene mutations, chromosomal aberrations, and the formation of micronuclei. In addition to the evidence supporting a mutagenic mode of action in test animals, alternative or additional hypothesized modes of action for hexavalent chromium carcinogenicity have not been demonstrated.

(2) *Is the hypothesized mode of action relevant to humans?* Mutagenicity is a well-established cause of carcinogenicity. The evidence discussed above demonstrates that hexavalent chromium is a mutagen in bacteria, yeast, cultured rodent and human cells, fruit flies, mice, and rats, supporting the presumption that it could also be a mutagen in humans. Moreover, several studies of exposed workers provide direct evidence of DNA damage by hexavalent chromium. In conclusion, the weight of evidence supports a mutagenic mode of action for hexavalent chromium carcinogenicity.

(3) *Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?* The mutagenic mode of action is considered relevant to all populations and lifestages. According to U.S. EPA's *Supplemental Guidance* (U.S. EPA, 2005b), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for hexavalent chromium carcinogenicity and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with the *Supplemental Guidance*. In addition, individuals with genetic polymorphisms conveying deficiencies in DNA repair capacity may have increased susceptibility to hexavalent chromium carcinogenicity.

4.7.3.5. *Mutagenic Across All Routes of Exposure*

As summarized previously, following inhalation exposures, hexavalent chromium has been shown to induce lung tumors in a number of human occupational studies, as well as tumors at or near the site of entry in animal studies. Evidence also exists, however, that ingested hexavalent chromium can reach the systemic circulation and affect tissues beyond those at or near the site of entry. In addition to hexavalent chromium toxicity in the lungs, it can be absorbed by the lung when inhaled and can then enter systemic circulation. Consistent with this evidence, DNA damage, micronucleus induction, and sister chromatid exchanges have been observed in circulating peripheral lymphocytes from workers exposed to inhalation concentrations as low as 7.5 and 24.9 $\mu g/m^3$ (Benova et al., 2002), and for durations of 4 months to 14 years (Gambelunghe et al., 2003), 0.5–18 years (Stella et al., 1982), 2–>20 years (Benova et al., 2002), or 4–25 years (Vaglenov et al., 1999). These studies indicate that, while tumor incidence following inhalation exposure to hexavalent chromium occurs primarily in the lungs, hexavalent chromium also has the capacity to damage DNA in other tissues at timepoints and concentrations relevant to human exposures.

EPA has concluded that hexavalent chromium is carcinogenic by a mutagenic mode of action. Considering the available oral and inhalation evidence for mutagenicity and subsequent carcinogenicity and that these events are capable of occurring in all cells, this mode of action is considered to be applicable to all routes of exposure.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No studies are available that address the possible adverse effects of hexavalent chromium in children. However, there is evidence that hexavalent chromium may act through a mutagenic mode of action. In accordance with the *Supplemental Guidance* (U.S. EPA, 2005b), the mutagenic mode of carcinogenic action for hexavalent chromium would indicate an increased carcinogenic susceptibility for early-life exposures. In addition, developmental toxicity also is of concern due to the mutagenicity of hexavalent chromium and the possibility for genetic damage to the germ cells of the F1 generation that could be transmitted to the F2 generation. The reproductive and developmental toxicity studies that have been conducted employing hexavalent chromium suggest that the developing fetus may be a target of toxicity, as well as male and female reproductive organs, which may result in a reduction in fertility.

4.8.2. Possible Gender Differences

The extent to which men and women differ in susceptibility to hexavalent chromium is unknown. However, animal data exist that imply a difference between males and females in their response to ingestion of hexavalent chromium. For example, in the NTP (2008) study, at the highest concentration administered (516 mg/L), female rats exhibited a higher incidence of tumors of the oral cavity than male rats (i.e., 11/48 [23%] vs. 7/50 [14%], respectively). The biological significance of this finding at lower doses and for other species, including humans, is unknown.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Two types of studies are available that provide information on the toxicological effects of ingested chromium in humans. The first type of study provides evidence of acute human health effects in individuals who accidentally or intentionally ingested high (fatal or near-fatal) doses of hexavalent chromium. The second type of study provides evidence of chronic human health effects (primarily cancer) in populations exposed unintentionally to food or drinking water containing high levels of hexavalent chromium over an extended time period. Because both types of studies provide little information on dose-response relationships and because the second type of study is primarily concerned with cancer as an outcome, these available human data are not useful for quantifying the risk of noncancer effects resulting from chronic exposure to hexavalent chromium.

In animals, the effects of subchronic oral exposure to hexavalent chromium have been evaluated in rats (NTP, 2007; Quinteros et al., 2007; Rafael et al., 2007; Acharya et al., 2001; Chopra et al., 1996; Vyskocil et al., 1993) and mice (NTP, 2007; Asmatullah and Noreen, 1999), and the effects of chronic oral exposure to hexavalent chromium have been evaluated in rats (NTP, 2008; MacKenzie et al., 1958), mice (NTP, 2008), and dogs (Anwar et al., 1961). In particular, the subchronic and chronic studies conducted by NTP (2008, 2007) provide the most useful dose-response data on the noncancer effects of oral hexavalent chromium exposure because of their comprehensive assessments of numerous toxicological endpoints at multiple dose levels. A number of other studies of reproductive and developmental toxicity of hexavalent chromium have been conducted in rats, mice, and rabbits, but typically at higher doses and for shorter durations than the NTP (2008, 2007) studies. All of these animal studies are summarized in Table 4-26.

Results from the NTP (2007) subchronic (i.e., 90-day) study identified several hexavalent chromium-induced noncancer effects, including hematological effects, hepatotoxicity, alterations in lipid metabolism, and histopathological changes in GI tissues and pancreatic and mesenteric lymph nodes. The most sensitive hexavalent chromium-induced noncancer effects were microcytic, hypochromic anemia, increased serum liver enzyme activities, and histopathological changes to the duodenum and pancreatic lymph nodes in rats; and histopathological changes in the duodenum in mice. In the 2-year toxicology and carcinogenicity study by NTP (2008), the most sensitive noncancer effects identified were histopathological changes to the liver, duodenum, and mesenteric lymph nodes in rats; and in the duodenum, mesenteric lymph nodes, and liver in mice. LOAELs of 1.7–3.1 mg hexavalent chromium/kg-day were identified by EPA

in the subchronic NTP (2007) study, and LOAELs of 0.24–0.77 mg hexavalent chromium/kg-day were identified by EPA in the chronic NTP (2008) study.

Other subchronic and chronic oral exposure studies of hexavalent chromium compounds do not provide suitable data for identifying points of departure (PODs) for RfD derivation because comprehensive toxicological evaluations were not conducted in these studies. In addition, interpretation of results from these studies was compromised because of the small number of animals evaluated, lack of a dose-response relationship, or inadequate reporting of results (see Table 4-26). Where LOAELs were identified based on examination of a limited set of endpoints (e.g., Acharya et al., 2001; Chopra et al., 1996), the LOAELs were higher than those identified in the chronic NTP (2008) bioassay.

Studies of reproductive and developmental toxicity indicate that hexavalent chromium exposure can affect reproductive organs, increase pre- and postnatal implantation loss, and cause reduced fetal weight and fetal abnormalities. In general, the NOAELs or LOAELs associated with reproductive and developmental effects are higher than those identified in the subchronic and chronic toxicity studies summarized in Table 4-26.

Thus, based on the comprehensive examination of endpoints and measurement of sensitive endpoints of toxicity, the bioassays by NTP (2008, 2007) were deemed the best candidates for use in deriving an oral RfD for hexavalent chromium. Specifically, five studies, three subchronic (i.e., one in rats and two in mice) (NTP, 2007) and two chronic (i.e., one in rats and one in mice) (NTP, 2008), were identified as candidate principal studies. The key results from these five studies are summarized below.

5.1.1.1. *Subchronic Studies*

NTP (2007) 90-day studies in rats and mice

In F344/N rats, sodium dichromate dihydrate was administered in drinking water to groups of males and females at five different concentrations for 90 days. Based on average water consumption rates, the mean effective doses of hexavalent chromium were estimated by NTP to be 0, 1.7, 3.5, 5.9, 11.2, and 20.9 mg/kg-day for both males and females. Results of this study identified a LOAEL in male and female rats of 1.7 mg hexavalent chromium/kg-day; a NOAEL was not identified because effects were observed at the lowest dose tested. This LOAEL was based on observations of microcytic, hypochromic anemia, increased serum liver enzyme activities, and histopathological changes to pancreatic lymph nodes (in males) and histopathological changes to the duodenum (in females) at daily doses $\geq$1.7 mg hexavalent chromium/kg-day.

In B6C3F$_1$ mice, groups of males and females were exposed to sodium dichromate dihydrate in drinking water for 90 days. Based on water consumption monitored throughout the study, NTP calculated average daily doses over the 90-day treatment duration of approximately 0, 3.1, 5.3, 9.1, 15.7, and 27.9 mg hexavalent chromium/kg-day for both males and females.

Based on histopathological changes (histiocytic cellular infiltration) in the duodenum in both sexes, a LOAEL of 3.1 mg hexavalent chromium/kg-day was identified for male and female mice; a NOAEL was not identified because the effects observed were at the lowest dose tested.

In a comparative 90-day drinking water study in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice, groups of each strain were exposed to three different concentrations of sodium dichromate dihydrate. Based on water consumption and body weights monitored throughout the study, NTP calculated average daily doses over the 90-day treatment duration of approximately 0, 2.8, 5.2, or 8.7 mg hexavalent chromium/kg-day for all strains. At the end of the study, similar effects were observed in all three strains. A LOAEL of 2.8 mg hexavalent chromium/kg-day was identified based on histopathological changes in the duodenum in B6C3F$_1$ mice (histiocytic cellular infiltration and diffuse epithelial hyperplasia), BALB/c mice (histiocytic cellular infiltration), and *am3*-C57BL/6 mice (diffuse epithelial hyperplasia); a NOAEL was not identified because effects seen were at the lowest dose tested.

5.1.1.2. *Chronic Studies*

NTP (2008) 2-year studies in rats and mice

In F344/N rats, groups of 50 males and females were administered sodium dichromate dihydrate in drinking water at four different concentrations for 2 years. Based on measured water consumption rates and body weights in rats, NTP estimated that male rats received time-weighted average doses of hexavalent chromium of 0.21, 0.77, 2.1, or 5.9 mg/kg-day, while female rats received 0.24, 0.94, 2.4, or 7.0 mg/kg-day of hexavalent chromium. This study identified NOAEL and LOAEL values for noncancer effects in male rats of 0.21 and 0.77 mg hexavalent chromium/kg-day, respectively, based on increased incidences of nonneoplastic histopathological changes to the liver (basophilic foci), duodenum (histiocytic cellular infiltrate), and mesenteric lymph nodes (histiocytic cellular infiltrate and hemorrhage). In female rats, a LOAEL for noncancer effects of 0.24 mg hexavalent chromium/kg-day was identified based on the increased incidence of chronic inflammation of the liver (observed in all treatment groups); a NOAEL was not identified because effects observed were at the lowest dose tested.

In B6C3F$_1$ mice, groups of 50 males and females were administered sodium dichromate dihydrate in drinking water at four different concentrations for 2 years. Based on measured amounts of water consumption and body weights in mice, NTP estimated that male mice received average doses of hexavalent chromium of 0.38, 0.91, 2.4, or 5.9 mg/kg-day, while female mice received 0.38, 1.4, 3.1, or 8.7 mg/kg-day of hexavalent chromium. This study identified a LOAEL for noncancer effects of 0.38 mg hexavalent chromium/kg-day in both male and female B6C3F$_1$ mice; a NOAEL value was not identified because effects seen were at the lowest dose administered. In males, the LOAEL was based on increased incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia) and mesenteric lymph nodes (histiocytic cellular infiltration); in females, the LOAEL was based on increased

incidences of histopathological changes to the duodenum (diffuse epithelial hyperplasia), mesenteric lymph nodes (histiocytic cellular infiltration), liver (histiocytic cellular infiltration), and pancreas (depletion of cytoplasmic zymogen granules).

The NTP (2008) study was of chronic duration (i.e., 2 years), involved the use of multiple dose groups, and included a comprehensive evaluation of multiple endpoints. Also, this bioassay used lower doses than the subchronic (90-day) studies also conducted by NTP (2007), and thus provided dose-response information at lower exposure levels than the 90-day studies. Additionally, the chronic NTP (2008) study was more sensitive, yielding lower LOAELs than the subchronic studies. Thus, the chronic NTP (2008) study was selected as the principal study.

As indicated, NTP (2008) observed several hexavalent chromium-induced noncancer effects in their chronic studies in rats and mice. Based on a comparison of LOAELs in rats and mice (see Table 4-26), the lowest LOAELs were observed for the following seven effects:

(1) Chronic liver inflammation in female rats,

(2) Histiocytic cellular infiltration in the liver of female mice,

(3) Diffuse epithelial hyperplasia in the duodenum of male mice,

(4) Diffuse epithelial hyperplasia in the duodenum of female mice,

(5) Histiocytic cellular infiltration in the mesenteric lymph nodes of male mice

(6) Histiocytic cellular infiltration in the mesenteric lymph nodes of female mice, and

(7) Cytoplasmic cellular alteration of acinar epithelial cells in the pancreas of female mice.

All of these effects occurred at the lowest doses tested (i.e., 0.24 mg/kg-day in female rats and 0.38 mg/kg-day in male and female mice), and were considered as possible critical effects for derivation of the RfD for hexavalent chromium. The incidences of these seven effects across all treatment groups in NTP (2008) are shown in Table 5-1.

Table 5-1. Incidence data for lesions in female F344/N rats and male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

Endpoint	Dose (mg hexavalent chromium/kg-d)				
	0	**0.24**	**0.94**	**2.4**	**7.0**
Female rats					
Liver: chronic inflammation	12/50	21/50[a]	28/50[b]	35/50[b]	39/50[b]
	Dose (mg hexavalent chromium/kg-d)				
	0	**0.38**	**0.91**	**2.4**	**5.9**
Male mice					
Duodenum: diffuse epithelial hyperplasia	0/50	11/50[b]	18/50[b]	42/50[b]	32/50[a]
Mesenteric lymph node: histiocytic cellular infiltration	14/47	38/47[b]	31/49[b]	32/49[b]	42/46[a]
	Dose (mg hexavalent chromium/kg-d)				
	0	**0.38**	**1.4**	**3.1**	**8.7**
Female mice					
Duodenum: diffuse epithelial hyperplasia	0/50	16/50[b]	35/50[b]	31/50[b]	42/50[b]
Mesenteric lymph node: histiocytic cellular infiltration	3/46	29/48[b]	26/46[b]	40/50[b]	42/50[b]
Liver: histiocytic cellular infiltration	2/49	15/50[b]	23/50[b]	32/50[b]	45/50[b]
Pancreas: acinus, cytoplasmic alteration	0/48	6/50[a]	6/49[a]	14/50[b]	32/50[b]

[a]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.
[b]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.

Source: NTP (2008).

5.1.2. Methods of Analysis—Including Models (PBPK, BMD, etc.)

To determine the specific endpoint for use in derivation of the RfD, all available dichotomous models in U.S. EPA's Benchmark Dose Software (BMDS), version 1.4.1, were fit to the incidence data for the seven selected endpoints (see Table 5-1) in female rats and male and female mice administered sodium dichromate dihydrate in drinking water for 2 years (NTP, 2008). The incidence data employed in the benchmark dose (BMD) modeling of these seven endpoints also are shown in Table 5-1. Doses (i.e., the benchmark dose [BMD_{10}] and the 95% lower confidence limit on the benchmark dose [$BMDL_{10}$]) associated with a benchmark response (BMR) of 10% extra risk were estimated by each model. In accordance with U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), a BMR of 10% is generally used in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across assessments.

Details of the BMD modeling conducted for each endpoint presented in Table 5-1 are provided in Appendix B. In general, model fit was assessed by a chi-square goodness-of-fit test (i.e., models with $p < 0.1$ failed to meet the goodness-of-fit criterion) and the Akaike's

Information Criterion (AIC) value (i.e., a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint). Of the models exhibiting adequate fit, the model yielding the lowest AIC value was selected as the best-fit model (as long as the BMDL estimates across the models exhibiting adequate fit were "sufficiently close"). If more than one model shared the lowest AIC, $BMDL_{10}$ values from these models were averaged to obtain a POD (U.S. EPA, 2000b).

For chronic liver inflammation in female rats with all dose groups included, only the log-logistic model provided an adequate fit, as assessed by the chi-square goodness-of-fit statistic, yielding BMD_{10} and $BMDL_{10}$ values of 0.22 and 0.14 mg hexavalent chromium/kg-day, respectively. For diffuse epithelial hyperplasia in the duodenum of male mice with all dose groups included, none of the dichotomous models in BMDS provided an adequate fit to the data (i.e., χ^2 *p*-value < 0.1). After dropping the high-dose group, the gamma, log-logistic, multistage, log-probit, quantal linear, and Weibull models provided adequate fits to the data (i.e., χ^2 *p*-value ≥ 0.1). As assessed by comparing AIC values, the multistage and quantal linear models provided the best fit, yielding BMD_{10} and $BMDL_{10}$ values of 0.16 and 0.13 mg hexavalent chromium/kg-day, respectively. For diffuse epithelial hyperplasia in the duodenum of female mice with all dose groups included, none of the dichotomous models in BMDS provided an adequate fit to the data (i.e., χ^2 *p*-value < 0.1). Only after dropping the two highest dose groups was an adequate fit achieved for any model. In this instance, all of the dichotomous models in BMDS, except the logistic and probit models, provided an adequate fit to the data (i.e., χ^2 *p*-value ≥ 0.1). As assessed by comparing the AIC values, the best fit was provided by several models (i.e., gamma, multistage, quantal linear, and Weibull), yielding BMD_{10} and $BMDL_{10}$ values of 0.12 and 0.09 mg hexavalent chromium/kg-day, respectively. For histiocytic cellular infiltration in the liver of female mice with all dose groups included, only the log-logistic model provided an adequate fit to the data (i.e., χ^2 *p*-value ≥ 0.1), yielding BMD_{10} and $BMDL_{10}$ values of 0.17 and 0.12 mg hexavalent chromium/kg-day, respectively. For cytoplasmic alteration of acinar epithelial cells of the pancreas in female mice, all of the dichotomous models in BMDS provided adequate fits to the data (i.e., χ^2 *p*-value ≥ 0.1). As assessed by comparing AIC values, the log-logistic model produced the best fit, yielding BMD_{10} and $BMDL_{10}$ values of 0.68 and 0.52 mg hexavalent chromium/kg-day, respectively. Finally, for lesions of the mesenteric lymph nodes (i.e., histiocytic cellular infiltration) in both male and female mice, none of the available dichotomous models in BMDS provided adequate fits to the data, even with the two highest doses dropped from the analysis; thus, data sets for these lesions were considered to be unsuitable for BMD modeling. Therefore, the LOAEL of 0.38 mg hexavalent chromium/kg-day for histiocytic cellular infiltration of the mesenteric lymph nodes in male and female mice serves as the candidate POD for this endpoint.

A summary of this BMD modeling information is presented in Table 5-2, and further details of this modeling are contained in Appendix B-1.

Table 5-2. Summary of BMD_{10} and $BMDL_{10}$ from the best fitting models for lesions of the liver, duodenum, mesenteric lymph nodes, and pancreas in female rats and male and female mice after exposure to sodium dichromate dihydrate in drinking water for 2 years (NTP, 2008)

Endpoint	Species/sex	Model	Number of doses	BMD[a] (mg/kg-d)	BMDL[a] (mg/kg-d)
Liver: chronic inflammation	Rat/female	Log-logistic	5	0.22	0.14
Duodenum: diffuse epithelial hyperplasia	Mouse/male	1-Degree polynomial multistage/quantal linear	4	0.16	0.13
Mesenteric lymph node: histiocytic cellular infiltration[b]	Mouse/male	–	–	–	–
Duodenum: diffuse epithelial hyperplasia	**Mouse/female**	**Gamma/multistage/quantal linear/Weibull**	**3**	**0.12**	**0.09**
Mesenteric lymph node: histiocytic cellular infiltration[b]	Mouse/female	–	–	–	–
Liver: histiocytic cellular infiltration	Mouse/female	Log-logistic	5	0.17	0.12
Pancreas: acinus, cytoplasmic alteration	Mouse/female	Log-logistic	5	0.68	0.52

[a]BMDs and BMDLs from dichotomous data are associated with a 10% extra risk; doses are in terms of mg hexavalent chromium/kg-d.
[b]None of the models provided an adequate fit to the data.

BMDL = lower confidence limit (95%) on the BMD

Source: ATSDR (2008).

The lowest $BMDL_{10}$ value of 0.09 mg hexavalent chromium/kg-day, based on the selection of the incidence of diffuse epithelial hyperplasia of the duodenum in female mice as the critical effect, was identified as the POD from which to derive the RfD for hexavalent chromium. As indicated in Section 4, due to its morphological similarity to adenoma, focal epithelial hyperplasia was classified as a preneoplastic lesion by NTP (2008), and so the possibility exists that diffuse epithelial hyperplasia may also represent a preneoplastic lesion. However, even though this possibility exists and thus this lesion may progress to cancer (i.e., adenoma) in some cases, the EPA considers the selection of this critical effect on which to base the derivation of the RfD (a noncancer endpoint) to be appropriate because definitive data on the progression of this particular lesion do not currently exist.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The following UFs were applied to the POD of 0.09 mg/kg-day, based on the incidence of diffuse epithelial hyperplasia of the duodenum in female mice from NTP (2008), to derive the RfD for hexavalent chromium.

- A UF of 10 was used to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability) because information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans.

- A UF of 10 was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability) because information is unavailable to predict potential variability in human susceptibility.

- A UF was not needed to account for extrapolation from subchronic-to-chronic exposure because a chronic study was used to derive the chronic RfD.

- A UF for LOAEL to NOAEL extrapolation was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR represented by a 10% extra risk of diffuse epithelial hyperplasia was selected under an assumption that it represents a minimal biologically significant change.

- A UF of 1 was used to account for database deficiencies. The toxicity of ingested hexavalent chromium has been extensively examined in a range of animal toxicology studies. The database for oral toxicity includes a chronic drinking water study in rats and mice, a chronic drinking water study in rats, a subchronic drinking water study in rats and mice, and a number of reproductive/developmental toxicity studies in monkeys, rabbits, rats, and mice. The reproductive toxicity database includes a continuous breeding study (NTP, 1997), in which F_0 and F_1 generation animals were exposed to hexavalent chromium in the diet, and the offspring of F_1 animals were evaluated on PND 21.

For this assessment, the RfD of 0.0009 or 9×10^{-4} mg/kg-day for hexavalent chromium was derived by dividing the $BMDL_{10}$ (or POD) of 0.09 mg/kg-day by a composite uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

5.1.4. Previous RfD Assessment

The previous RfD assessment for hexavalent chromium was completed in September 1998. The previous RfD was based on a NOAEL identified from a 1-year drinking water study in rats in which animals were exposed to hexavalent chromium (as potassium chromate) at a dose of 2.5 mg/kg-day (MacKenzie et al., 1958). No toxicity was reported in these animals at this dose, resulting in identification of a NOAEL of 2.5 mg/kg-day, the only dose administered in

the study, as the POD. A composite uncertainty factor of 300 (10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for subchronic to chronic extrapolation) and a modifying factor of 3 (to account for concerns raised by the epidemiology study of Zhang and Li, 1987) were applied to this POD to yield an oral RfD of 3×10^{-3} mg/kg-day.

5.2. UNCERTAINTIES IN THE ORAL REFERENCE DOSE

The following discussion identifies uncertainties associated with the RfD for hexavalent chromium. As presented above, an RfD of 9×10^{-4} mg/kg-day was derived based on the incidence of diffuse epithelial hyperplasia of the duodenum in female mice from a 2-year drinking water study (NTP, 2008). UFs were applied to the POD, a $BMDL_{10}$ generated through BMD modeling. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to humans with varying susceptibilities.

An adequate range of animal toxicology data is available for the hazard assessment of hexavalent chromium via ingestion, as described previously in Chapter 4. The database of oral toxicity studies includes a chronic drinking water study in rats and mice, a chronic drinking water study in rats, a subchronic drinking water study in rats and mice, and several reproductive/developmental toxicity studies in monkeys, rabbits, rats, and mice. Toxicity associated with oral exposure to hexavalent chromium is observed in the liver, GI tract, and reproductive organs, with the liver and GI tract being the most sensitive target organs.

Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 2-year drinking water study in F344/N rats and $B6C3F_1$ mice (NTP, 2008) and increased incidence of diffuse epithelial hyperplasia in the duodenum of female mice as the principal study and critical effect, respectively, for deriving the RfD for hexavalent chromium.

The selection of the BMD model for identifying the POD does not lead to significant uncertainties since benchmark effect levels were within the range of the experimental data. However, the selected models do not represent all possible models one might fit, and other models could be selected to yield more extreme results, both higher and lower than those included in this assessment.

Animal-to-human extrapolation yields further uncertainties. The effect and the magnitude of this effect associated with the dose at the POD in mice are extrapolated to humans. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible for the toxicity observed following oral exposure to hexavalent chromium. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals

and humans. Accordingly, a 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD.

Heterogeneity among humans is another area of uncertainty. In the absence of hexavalent chromium-specific data on variation in human response, a factor of 10 was used in the derivation of the RfD. Human variation may be larger or smaller than this 10-fold factor; however, hexavalent chromium-specific data to examine the potential magnitude of over- or underestimation are unavailable.

5.3. ORAL CANCER ASSESSMENT

5.3.1. Choice of Study/Data—with Rationale and Justification

Several epidemiology studies have examined the association between oral exposure to environmental hexavalent chromium and cancer in populations that resided near sources of industrial waste containing hexavalent chromium compounds, including studies of populations in Liaoning Province, China (Kerger et al., 2009; Beaumont et al., 2008; Zhang and Li, 1997, 1987, 1980), Kings County/San Bernardino County, California (Fryzek et al., 2001), Nebraska (Bednar and Kies, 1991), and Glasgow, United Kingdom (Eizaguirre-Garcia et al., 2000, 1999). The Liaoning Province studies provide some evidence of an excess risk of mortality from stomach cancer; however, because of various limitations, including limited characterization of exposure, the Liaoning Province studies are not considered adequate for dose-response analysis.

The NTP rodent bioassay, in which F344/N rats and $B6C3F_1$ mice were administered sodium dichromate dihydrate, a hexavalent chromium compound, in drinking water for 2 years (NTP, 2008), was selected as the basis for deriving the oral cancer slope factor (CSF) for hexavalent chromium. This bioassay was selected for dose-response assessment because it is a well-conducted lifetime animal study of hexavalent chromium carcinogenicity via ingestion (see detailed summary of the study in Section 4.2.2). No other adequate studies of hexavalent chromium carcinogenicity by ingestion are available.

5.3.2. Dose-Response Data

The dose-response data considered in the derivation of the CSF for hexavalent chromium were the incidences of benign and malignant tumors in rat oral mucosa and mouse small intestine observed in the NTP (2008) bioassay.

Incidence data for neoplastic lesions of the oral cavity in male and female rats exposed to sodium dichromate dihydrate in drinking water for 2 years are summarized in Table 4-15. Neoplasms observed in the oral cavity of treated rats were squamous cell carcinoma of the oral mucosa (both sexes), squamous cell papilloma of the oral mucosa (males only), squamous cell carcinoma of the tongue (both sexes), and squamous cell papilloma of the tongue (both sexes). The incidences of squamous cell carcinoma of the oral mucosa (13.6%) and of combined squamous cell papilloma or carcinoma (15.7%) of the oral mucosa were statistically significantly

increased (at $p < 0.05$) in male rats treated with 5.9 mg/kg-day hexavalent chromium (the highest dose tested) compared with controls. The incidences of squamous cell carcinoma of the oral mucosa (23.9%) and of combined squamous cell carcinoma of the oral mucosa or tongue (23.9%) were statistically significantly increased (at $p < 0.05$) in female rats treated with 7.0 mg hexavalent chromium/kg-day (the highest dose tested) compared with controls. The incidences of other neoplastic lesions of the oral cavity were not statistically significantly increased in any treatment group in male or female rats compared with controls, although the incidence of squamous cell carcinoma of the oral mucosa in female rats in the penultimate (2.4 mg/kg-day) dose group (4.6%) exceeded that of historical controls (i.e., 0/300 in drinking water studies; 5/1,400 [0.4%] by all routes of exposure). Other neoplasms observed in treated rats included pancreatic acinar adenomas and benign pheochromocytomas in males and mononuclear cell leukemias in females (see Table 4-16); however, the incidence of these neoplasms did not exhibit dose-dependence. Thus, NTP (2008) concluded that evidence of a relationship between neoplastic changes in tissues other than the oral cavity and exposure to sodium dichromate dihydrate was equivocal. In summary, exposure of rats to sodium dichromate dihydrate in drinking water for 2 years resulted in a significant increase in squamous epithelial neoplasms of the oral mucosa and tongue at the highest exposure levels (average daily doses of 5.9 and 7.0 mg hexavalent chromium/kg-day in males and females, respectively), but not at the three lower exposure levels. The incidences of squamous cell papillomas or carcinomas in the oral cavity of male and female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years in the NTP (2008) study are presented in Table 5-3 (for male rats) and Table 5-4 (for female rats).

Table 5-3. Incidences of squamous cell papillomas or carcinomas in the oral cavity of male F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years

Sodium dichromate dihydrate concentration (mg/L)	Estimated daily intake of hexavalent chromium[a] (mg/kg-d)	Incidence of squamous cell papillomas or carcinomas[b]
0	0	0/50 (0%)
14.3	0.21	1/50 (2%)
57.3	0.77	0/49 (0%)
172	2.1	0/50 (0%)
516	5.9	7/49 (14.5%)[c]

[a]Intakes were reported by NTP (2008) based on drinking water intakes and mean body weights observed during the study.
[b]Number of animals with lesion/number of animals examined. Incidence estimates include all animals that were examined for oral tumors unadjusted for survival.
[c]Statistically significantly elevated above control at $p < 0.05$ using Fisher's exact test.

Source: NTP (2008).

Table 5-4. Incidences of squamous cell papillomas or carcinomas in the oral cavity of female F344/N rats exposed to sodium dichromate dihydrate in drinking water for 2 years

Sodium dichromate dihydrate concentration (mg/L)	Estimated daily intake of hexavalent chromium[a] (mg/kg-d)	Incidence of squamous cell papillomas or carcinomas[b]
0	0	1/50 (2%)
14.3	0.24	1/50 (2%)
57.3	0.94	0/50 (0%)
172	2.4	2/50 (4%)
516	7.0	11/50 (22%)[c]

[a]Intakes were reported by NTP (2008) based on drinking water intakes and mean body weights observed during the study.
[b]Number of animals with lesion/number of animals examined. Incidence estimates include all animals that were examined for oral tumors unadjusted for survival.
[c]Statistically significantly elevated above control at $p < 0.05$ using Fisher's exact test.

Source: NTP (2008).

Also from the NTP (2008) study, incidence data for neoplastic lesions of the small intestine in male and female $B6C3F_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years are summarized in Table 4-19. In male mice, statistically significant increases ($p < 0.05$) were observed in the incidences of adenomas or carcinomas combined in the small intestine (duodenum, jejunum, and ileum) at hexavalent chromium doses ≥2.4 mg/kg-day (i.e., at the two highest doses tested). Furthermore, significant positive trends were observed in the incidences of duodenal adenomas, duodenal carcinomas, jejunal adenomas, small intestine

adenomas, small intestine carcinomas, and small intestine adenomas or carcinomas combined in male mice. In female mice, statistically significant increases ($p < 0.05$) were observed in the incidences of duodenal adenomas, small intestine adenomas, and small intestine adenomas or carcinomas combined at hexavalent chromium doses ≥3.1 mg/kg-day (i.e., at the two highest doses tested). Furthermore, significant positive trends were observed in the incidences of duodenal adenomas, duodenal carcinomas, jejunal adenomas, small intestine adenomas, and small intestine adenomas or carcinomas combined in female mice. No other statistically or biologically significant increases in neoplasms were observed in other tissues.

In summary, exposure of $B6C3F_1$ mice to sodium dichromate dihydrate in drinking water for 2 years resulted in statistically significant increases in the incidences of neoplasms of the small intestine in males and females at hexavalent chromium doses ≥2.4 and ≥3.1 mg/kg-day, respectively. The incidences of adenomas and carcinomas combined in the small intestine of male and female $B6C3F_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years are summarized in Tables 5-5 and 5-6, respectively. In evaluating the tumor incidences in rats and mice, the mouse was determined to be the most sensitive species because tumor incidences were statistically significantly elevated at lower doses and a greater response was exhibited by the mice at the two highest doses. Therefore, the mouse tumor incidence data were used as the basis for the oral CSF derived employing BMD modeling.

Table 5-5. Incidences of adenomas and carcinomas combined in the small intestine of male $B6C3F_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

Sodium dichromate dihydrate concentration (mg/L)	Estimated daily intake of hexavalent chromium[a] (mg/kg-d)	Incidence of adenomas or carcinomas[b]
0	0	1/49 (2%)
14.3	0.38	3/49 (6.1%)
28.6	0.91	2/49 (4.1%)
85.7	2.4	7/50 (14%)[c]
257.4	5.9	20/48 (41.7%)[c]

[a]Intakes were reported by NTP (2008) based on drinking water intakes and mean body weights observed during the study.
[b]Calculated from reported percentages of mice with adenomas or carcinomas. Incidence estimates included all animals that were examined for intestinal tumors and survived for at least 451 days. In each of the control and first two dose groups, one animal died prior to day 451. In the high-dose group, two animals died prior to day 451. None of these animals were found to have intestinal adenomas or carcinomas at the time of death.
[c]Statistically significantly elevated above control at $p < 0.05$ using Fisher's exact test.

Source: NTP (2008).

Table 5-6. Incidences of adenomas and carcinomas combined in the small intestine of female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years

Sodium dichromate dihydrate concentration (mg/L)	Estimated daily intake of hexavalent chromium[a] (mg/kg-d)	Incidence of adenomas or carcinomas[b]
0	0	1/49 (2%)
14.3	0.38	1/50 (2%)
57.3	1.4	4/49 (8.2%)
172	3.1	17/49 (34.7%)[c]
516	8.7	22/49 (44.9%)[c]

[a]Intakes were reported by NTP (2008) based on drinking water intakes and mean body weights observed during the study.
[b]Calculated from reported percentages of mice with adenomas or carcinomas. Incidence estimates included all animals that were examined for intestinal tumors and survived for at least 451 days. In all of the dose groups except the low-dose group, one animal died prior to day 451. None of these animals were observed to have intestinal adenomas or carcinomas at the time of death.
[c]Statistically significantly elevated above control at $p < 0.05$ using Fisher's exact test.

Source: NTP (2008).

5.3.3. Dose Adjustments and Extrapolation Method(s)

U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical be determined by what is known about the mode of action of that chemical, and how this mode of action impacts the shape of the dose-response curve at low doses. According to the Cancer Guidelines, the dose response is generally considered to be linear in the low-dose range when evidence supports a mutagenic mode of action for a chemical because of its DNA reactivity and direct mutagenic activity. A linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with hexavalent chromium exposure due to the mutagenic mode of action of this chemical.

In order to derive an oral CSF, BMD modeling was carried out using U.S. EPA's BMDS (U.S. EPA, 2000b). U.S. EPA's BMDS offers several possible mathematical dose-response functions for use with dichotomous data including logistic, gamma, Weibull, quantal linear, probit, and multistage models. For this assessment, EPA relied on the results obtained from the multistage model only, as this is the model preferred by the Agency for conducting cancer dose-response assessments. In applying the BMD approach to the derivation of a CSF, the standard procedure is to calculate a lower 95% confidence bound on the dose corresponding to the BMR, where the BMR is typically set at 10% extra risk. This lower confidence bound is referred to as the $BMDL_{10}$. The CSF is then calculated by dividing the BMR by the $BMDL_{10}$ and then converting this slope value to human equivalents.

In estimating the CSF, the incidence of neoplasms in the small intestine of mice was employed, as this species was deemed to be more sensitive than the rat. Only animals that survived for at least 451 days, the time until appearance of the first tumor, were considered at risk for tumor development. Consequently, the incidence estimates included all animals that were examined for intestinal tumors and survived for at least 451 days (see Tables 5-5 and 5-6). The BMD modeling results for the incidence of neoplasms in the small intestine of male and female mice are shown in Appendix B-2. For male mice, the two-stage multistage model exhibited the best fit to the data yielding a slope of 0.09 $(mg/kg\text{-}day)^{-1}$. For female mice, the two-stage multistage model also exhibited the best fit to the data yielding a slope of 0.10 $(mg/kg\text{-}day)^{-1}$.

In order to estimate an oral CSF, these slopes were converted to human equivalents. For this conversion, body weight to the ¾ power scaling was used, where the time-weighted average male and female mouse body weights of controls (i.e., 50 and 53 g, respectively) were employed, along with an assumed human body weight of 70 kg. The mouse body weights were taken from the NTP (2008) study report. The following equation was then used to convert the slopes derived from the BMD modeling to oral CSFs expressed in human equivalents:

$$\text{Slope} \times (W_H/W_A)^{0.25} = \text{CSF}$$

where
W_H = animal body weight (kg)
W_A = human body weight (kg)

Using the above equation, the CSFs resulting from the fitting of the two-stage multistage model in BMDS to the incidence of neoplasms in the small intestine of male or female mice were 0.5 and 0.6 $(mg/kg\text{-}day)^{-1}$, respectively, expressed in human equivalents.

5.3.4. Oral Slope Factor

The CSF values based on the incidence of small intestine tumors in male and female mice are similar (i.e., 0.5 $[mg/kg\text{-}day]^{-1}$ for males and 0.6 $[mg/kg\text{-}day]^{-1}$ for females). Given the poorer fit of the multistage model to the female mouse data, a CSF estimate based on the male mouse data was considered to be associated with less uncertainty. Therefore, the CSF of 0.5 $(mg/kg\text{-}day)^{-1}$, based on the incidence of neoplasms in the small intestine of male mice, was selected as the most appropriate CSF for hexavalent chromium.

5.3.5. Application of ADAFs

Because a mutagenic mode of action for hexavalent chromium carcinogenicity is sufficiently supported in laboratory animals and is relevant to humans (see Section 4.6.3.4), and in the absence of chemical-specific data to evaluate differences in age-specific susceptibility,

increased early-life susceptibility to hexavalent chromium is assumed and ADAFs should be applied, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The oral slope factor of 0.5 $(mg/kg\text{-}day)^{-1}$, calculated from data applicable to adult exposures, does not reflect presumed early-life susceptibility to this chemical. Example calculations for estimating cancer risks based on age at exposure are provided in Section 6 of the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

The *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* establishes ADAFs for three specific age groups. The current ADAFs and their corresponding age groups are 10 for exposed individuals <2 years old, 3 for exposed individuals 2 to <16 years old, and 1 for exposed individuals ≥16 years old (U.S. EPA, 2005b). The 10- and 3-fold adjustments to the slope factor are to be combined with age-specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposures to hexavalent chromium.

To illustrate the use of the ADAFs established in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), sample calculations are presented for three exposure duration scenarios, including full lifetime, assuming that the exposure rate to hexavalent chromium remains constant at an average daily dose of 0.0001 mg hexavalent chromium/kg-day (Table 5-7). This average daily dose of 0.0001 mg hexavalent chromium/kg-day is being used here for illustrative purposes only to demonstrate how to apply ADAFs. In practice, actual exposure information specific to the situation under consideration should be used.

Table 5-7. Application of ADAFs for a 70-year exposure to 0.0001 mg hexavalent chromium/kg-day from ages 0 to 70

Age group	ADAF	Slope factor (per mg/kg-d)	Average daily dose (mg/kg-d)	Duration adjustment	Partial risk
0–<2 yrs	10	0.5	0.0001	2 yrs/70 yrs	1×10^{-5}
2–<16 yrs	3	0.5	0.0001	14 yrs/70 yrs	3×10^{-5}
≥16 yrs	1	0.5	0.0001	54 yrs/70 yrs	4×10^{-5}
				Total risk	8×10^{-5}

Note that the partial risk for each age group is the product of the values in columns 2–5 (e.g., $10 \times 0.5 \times 0.0001 \times 2/70 = 0.00001$ for exposures from age 0 to <2 years), and the total risk is the sum of the partial risks. Thus, a 70-year risk estimate for a constant average daily dose of 0.0001 mg/kg-day starting at birth is 0.00008 or 8×10^{-5}.

If calculating the cancer risk for a 30-year exposure to a constant average daily dose of 0.0001 mg hexavalent chromium/kg-day from ages 0 to 30 years, the duration adjustments would

be 2/70, 14/70, and 14/70, and the partial risks would be 0.00001, 0.00003, and 0.00001, resulting in a total risk estimate of 0.00005 or 5×10^{-5}.

If calculating the cancer risk for a 30-year exposure to a constant average daily dose of 0.0001 mg hexavalent chromium/kg-day from ages 20 to 50 years, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks would be 0, 0, and 0.00002, resulting in a total risk estimate of 0.00002 or 2×10^{-5}.

5.3.6. Uncertainties in Cancer Risk Values

As in most risk assessments, extrapolation of data from experimental animals to estimate potential lifetime cancer risks to human populations from exposure to hexavalent chromium yields uncertainties. Some of these uncertainties can be evaluated for their quantitative impact on the final result, while for others, only their qualitative impact can be assessed. The principal uncertainties in the assessment of the cancer risk from exposure to hexavalent chromium are summarized below in Table 5-8, and discussed in more detail in the following text.

Table 5-8. Summary of uncertainties in the cancer risk assessment for hexavalent chromium

Consideration/ approach	Impact on oral slope factor	Decision	Justification
Low-dose extrapolation procedure	Alternatives could ↓ or ↑ CSF by an unknown extent	Multistage model used to determine POD, linear low-dose extrapolation from POD	A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with hexavalent chromium exposure consistent with a mutagenic mode of carcinogenic action.
Cross-species scaling	Alternatives could ↓ or ↑ CSF (e.g., sixfold ↓ [scaling by BW] or ↑ twofold [scaling by $BW^{2/3}$])	$BW^{3/4}$ (default approach)	In the absence of hexavalent chromium-specific information on interspecies differences in toxicokinetics, the default scaling factor of $BW^{3/4}$ was used to calculate equivalent cumulative exposures for estimating equivalent human risks (U.S. EPA, 1992).
Statistical uncertainty at POD	↓ CSF 25% if maximum likelihood estimation (i.e., BMD_{10}) used rather than lower bound ($BMDL_{10}$) for POD	BMDL (default approach for calculating reasonable upper bound CSF)	Size of bioassay results in sampling variability; lower bound is 95% CI on administered dose.
Species/gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Male mouse tumors (adenomas or carcinomas of the small intestine)	It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. Generally, direct site concordance is not assumed; consistent with this view, some human tumor types are not found in rodents and rat and mouse tumor types also differ.
Human relevance of rodent tumor data	Lack of human relevance of tumor data would ↓ CSF	Tumors with significant dose-response considered for estimating potential human cancer response	Hexavalent chromium is judged to be carcinogenic through a mutagenic mode of action and is a multisite carcinogen in rodents; therefore, the carcinogenicity observed in rodent studies is assumed to be relevant to human exposure.
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data are available to support the range of human variability/sensitivity to hexavalent chromium.

Choice of low-dose extrapolation approach. The mode of action is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear, low-dose extrapolation approach was used to estimate human carcinogenic risk associated with hexavalent chromium exposure consistent with a hypothesized mutagenic mode of carcinogenic action of hexavalent chromium (U.S. EPA, 2005a).

The multistage model was used to model the tumor incidence data because this is the model preferred by the Agency for conducting cancer dose-response assessments; however, it is unknown how well this model or the linear low-dose extrapolation predicts low-dose risks for hexavalent chromium. The selected model does not represent all possible models one might fit, and other models could conceivably be selected to yield more extreme results consistent with the observed data, both higher and lower than those included in this assessment.

Cross-species scaling. The default cross-species scaling factor ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between rodent species and humans, consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Because it is unknown whether there are differences in the pharmacokinetic pathways in animals and humans following hexavalent chromium exposure, it is not possible to estimate the magnitude of the uncertainty in the use of this default beyond that associated with other choices for default cross-species scaling factors (e.g., $BW^{2/3}$ or BW^{1}).

Statistical uncertainty at the POD. Measures of statistical uncertainty require assuming that the underlying model and associated assumptions are valid for the data under consideration. For the multistage model applied to the incidence of male mice GI tract tumors, there is a reasonably typical degree of uncertainty at the 10% extra risk level (the POD for linear low-dose extrapolation). That is, the $BMDL_{10}$ for male mice is approximately 25% lower than the BMD_{10}.

Choice of species/gender. The oral CSF for hexavalent chromium was quantified using the tumor incidence data for mice, which were thought to be more sensitive than rats to the carcinogenicity of hexavalent chromium. While tumor responses in the mouse were higher than those of rats at a comparable dose level, suggesting greater sensitivity of the mouse, it is unknown whether this higher sensitivity would be maintained at lower exposures.

Relevance to humans. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state that site concordance is not a prerequisite for evaluating the implications of animal study results for humans. Chemicals that are mutagenic and cause tumors at multiple sites in animals are likely relevant to human carcinogenesis. Hexavalent chromium is thought to be carcinogenic through a mutagenic mode of action and is a multisite carcinogen in rodents. Considering all of the available information, the carcinogenicity observed in rodent studies is considered relevant to human exposure. In addition, the concordance of the alimentary system tumors across rats and mice lends strength to the concern for human carcinogenic potential.

Human population variability. The extent of inter-individual variability in response to hexavalent chromium is unknown. Although a mutagenic mode of action would indicate increased early-life susceptibility, the data exploring whether there is differential sensitivity to hexavalent chromium carcinogenicity across life stages are unavailable. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. The uncertainties associated with this lack of data and knowledge about human variability can, at present, only be considered in

qualitative terms; however, EPA has developed ADAFs to quantitatively account for some of the potential differences in age-dependent response to carcinogens with a mutagenic mode of action. ADAFs are to be applied to the CSF for hexavalent chromium when assessing cancer risks in exposed populations composed of individuals <16 years old (U.S. EPA, 2005b). More specific guidance in applying these ADAFs was provided in Section 5.3.5.

5.3.7. Previous Cancer Assessment

The previous IRIS assessment for hexavalent chromium was posted to the IRIS database in 1998. In that assessment, EPA concluded that the oral carcinogenicity of hexavalent chromium could not be determined (and was thus classified as Group D) because no data were located in the available literature that suggested that hexavalent chromium is carcinogenic by the oral route of exposure. Therefore, no oral CSF was derived.

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

6.1. HUMAN HAZARD POTENTIAL

Hexavalent chromium compounds are a group of substances that contain chromium in the hexavalent or +6 oxidation state. As a class, hexavalent chromium compounds are strong oxidizing agents, and thus, it is rare to find hexavalent chromium naturally occurring in the environment because it is readily reduced to trivalent chromium (i.e., chromium in the +3 oxidation state) by organic matter. However, hexavalent chromium compounds released to the environment by anthropogenic sources may persist in natural waters and soils that contain low amounts of organic matter. Major uses or former uses of hexavalent chromium compounds include metal plating, manufacture of pigments and dyes, corrosion inhibitors, chemical synthesis, refractory production, leather tanning, and wood preservation. Individuals may be exposed to hexavalent chromium compounds through ingestion of drinking water or contact with soils or other media contaminated with these substances.

Toxicokinetic studies in humans, mice, and rats have examined the absorption, distribution, metabolism, and elimination of hexavalent chromium compounds. Hexavalent chromium can be absorbed via oral, inhalation, or dermal routes of exposure in humans and laboratory animals. For this toxicological review, however, the focus is on the toxicokinetics of hexavalent chromium following ingestion. Once ingested, hexavalent chromium compounds can interact with endogenous fluids and other organic matter in the GI tract, resulting, to some extent, in the reduction of hexavalent chromium to trivalent chromium. This process, whereby hexavalent chromium is reduced to trivalent chromium in the GI tract, is termed “extracellular” reduction. The extent of absorption of ingested hexavalent chromium appears to be determined by both the solubility of the hexavalent chromium compound ingested and how rapidly hexavalent chromium is reduced to trivalent chromium in the GI tract, as trivalent chromium does not diffuse readily across cell membranes. Hexavalent chromium can easily cross cell membranes due to its ability to use existing nonspecific sulfate and phosphate anion transport mechanisms.

Ingested hexavalent chromium is distributed throughout the body. Liver, kidney, spleen, and bone are the primary sites of chromium distribution. Once inside the cell, hexavalent chromium is reduced to trivalent chromium, either enzymatically or nonenzymatically. This process is called “intracellular” reduction to distinguish it from the extracellular process described above. This intracellular reduction yields such reactive intermediates as chromium(V) and chromium(IV). These reactive intermediates, along with oxygen radicals generated during this intracellular reduction, can indirectly damage DNA. In addition, trivalent chromium, the

final product of the intracellular reduction of hexavalent chromium, can form adducts with a number of macromolecules, including DNA.

Hexavalent chromium is eliminated primarily in the urine as trivalent chromium. Chromium can also be eliminated in hair, nails, and breast milk. There does not appear to be a gender difference in the toxicokinetics of hexavalent chromium, and inter-individual variability in the presystemic reduction and subsequent absorption and elimination may be primarily driven by differences in gastric contents and intervals between meals.

Two PBPK models have been developed for hexavalent and trivalent chromium in rats and humans (O'Flaherty et al., 2001; O'Flaherty, 1996, 1993). The inclusion of trivalent chromium in the model allows for the use of trivalent chromium exposure time course data to aid in parameterization of chromium elimination and to evaluate the ability of the model to predict elimination of hexavalent chromium as trivalent chromium. However, neither the rat nor human version of the model in its present form has been subjected to formal computerized optimization of parameter values.

Two types of studies provide information on the toxicological effects in humans resulting from exposure to ingested hexavalent chromium. In the first type of study, acute human health effects have been observed following oral ingestion of hexavalent chromium in individuals accidentally or intentionally ingesting high (fatal or near-fatal) doses of hexavalent chromium. These studies are not particularly useful for establishing dose-response relationships. In the second type of study, chronic human health effects have been reported in human populations exposed unintentionally to elevated levels of hexavalent chromium in food or drinking water over an extended time period. Human studies of possible associations between oral exposure to hexavalent chromium and cancer are limited to a few epidemiology studies in which health outcomes (primarily cancer) were evaluated among populations that were exposed to drinking water contaminated with hexavalent chromium in Liaoning Province, China (Kerger et al., 2009, Beaumont et al., 2008; Zhang and Li, 1997, 1987), Kings County/San Bernardino County, California (Fryzek et al., 2001; Bick et al., 1996), Nebraska (Bednar and Kies, 1991), and Glasgow, United Kingdom (Eizaguirre-Garcia et al., 2000, 1999). Analyses of data collected from the JinZhou area of Liaoning Province, China, where groundwater, surface water, and agricultural soils were heavily contaminated with chromium derived from hexavalent chromium production (e.g., 0.001–20 mg chromium/L in residential well water), provide evidence of an excess risk of mortality from stomach cancer from 1970 to 1978 in residents of the area, relative to the reference populations in the province (four other areas in Liaoning Province, and the total population of the province) (Beaumont et al., 2008). EPA concluded that the exposure-response analyses presented by Zhang and Li (1997), Beaumont et al. (2008), and Kerger et al. (2009) are not based on the quality of data that is needed to support a conclusion regarding the presence or absence of a dose-response among the observed cancer rates in these villages. The other epidemiologic studies did not find a significant correlation between hexavalent chromium

concentrations in drinking water (or proximity to the source of hexavalent chromium soil contamination) and cancer.

In animals, the effects of subchronic oral exposure to hexavalent chromium have been evaluated in rats (NTP, 2007; Quinteros et al., 2007; Rafael et al., 2007; Acharya et al., 2001; Chopra et al., 1996; Vyskocil et al., 1993) and mice (NTP, 2007; Asmatullah and Noreen 1999), and the effects of chronic oral exposure to hexavalent chromium have been evaluated in rats (NTP, 2008, MacKenzie et al., 1958), mice (NTP, 2008; Borneff et al., 1968), and dogs (Anwar et al., 1961). The studies conducted by the NTP (2008, 2007) provide dose-response data on the effects of oral hexavalent chromium exposure based on a comprehensive assessment of toxicological endpoints. The EPA used NTP (2008, 2007) to identify LOAELs and NOAELs in rats and mice for subchronic and chronic exposure durations. Results from the NTP (2007) subchronic study identified several hexavalent chromium-induced noncancer effects, including hematological effects, hepatotoxicity, alterations in lipid metabolism, and histopathological changes in GI tissues and pancreatic and mesenteric lymph nodes. The most sensitive hexavalent chromium-induced noncancer effects in rats were microcytic, hypochromic anemia, increased serum liver enzyme activities, and histopathological changes to the duodenum and pancreatic lymph nodes; in mice, the most sensitive noncancer effect was histopathological changes in the duodenum. The most sensitive noncancer effects in the NTP (2008) 2-year toxicology and carcinogenicity study were histopathological changes to the liver, duodenum, and mesenteric lymph nodes in rats; and in the duodenum, mesenteric lymph nodes, and liver in mice.

A number of animal studies have also evaluated the reproductive/developmental toxicity of hexavalent chromium via the oral route of exposure. Collectively, these studies provide evidence that oral exposure to hexavalent chromium compounds produces reproductive effects, including histopathological changes to reproductive organs in males (Aruldhas et al., 2006, 2005, 2004; Li et al., 2001; Chowdhury and Mitra, 1995; Zahid et al., 1990) and females (Murthy et al., 1996); alterations in sperm, including decreased count, decreased motility, and abnormal morphology (Subramanian et al., 2006; Yousef et al., 2006; Li et al., 2001; Zahid et al., 1990); decreased plasma testosterone levels (Yousef et al., 2006; Chowdhury and Mitra, 1995); increased estrous cycle length (Kanojia et al., 1998, 1996; Murthy et al., 1996); changes in mating behavior and decreased fertility in males (Bataineh et al., 1997); and adverse reproductive outcomes, including decreased numbers of live fetuses and implantations, and increased numbers of resorptions and pre- and postimplantation losses (Bataineh et al., 2007; Elsaieed and Nada, 2002; Kanojia et al., 1998, 1996; Elbetieha and Al-Hamood, 1997; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989). Developmental effects observed have included decreased fetal weight and length (Elsaieed and Nada, 2002; Kanojia et al., 1998; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989); external (subdermal hemorrhage and tail malformations) and skeletal abnormalities (decreased ossification) (Elsaieed and Nada, 2002; Kanojia et al.,

1998, 1996; Junaid et al., 1996a, b, 1995; Trivedi et al., 1989); and delayed sexual maturation and function in female offspring (Banu et al., 2008; Al-Hamood et al., 1998). In contrast to results of the above studies, effects were not observed in dietary exposure studies conducted by NTP that investigated the potential for hexavalent chromium to produce effects on male reproductive organs in rats and mice (NTP, 1996a, b) and on reproductive outcomes in a continuous breeding study in mice (NTP, 1997). The reasons for these inconsistent results are not readily apparent, as daily dose ranges evaluated in the NTP studies overlapped with those used in other studies showing hexavalent chromium-induced adverse reproductive effects. The most sensitive noncancer effects observed in the 2-year chronic study by NTP (2008), in general, occurred at lower doses than the reproductive or developmental effects.

In regard to carcinogenic effects, exposure of rats to sodium dichromate dihydrate in drinking water for 2 years resulted in a significant increase in squamous epithelial neoplasms of the oral mucosa and tongue at the highest exposure level (average daily doses of 5.9 and 7.0 mg hexavalent chromium/kg-day in males and females, respectively), but not at the three lower exposure levels (NTP, 2008). Exposure of $B6C3F_1$ mice to sodium dichromate dihydrate in drinking water for 2 years resulted in significant increases in the incidences of neoplasms of the small intestine in males and females at doses ≥2.4 and ≥3.1 mg hexavalent chromium/kg-day, respectively. NTP (2008) concluded that results from these studies provide clear evidence of carcinogenic activity of sodium dichromate dihydrate in male and female F344/N rats based on increased incidences of squamous cell neoplasms of the oral cavity and clear evidence of carcinogenic activity of sodium dichromate dihydrate in male and female $B6C3F_1$ mice based on increased incidences of neoplasms of the small intestine.

The potential mutagenicity of hexavalent chromium has been studied extensively. Although study results vary with specific test systems, experimental conditions, and hexavalent chromium compounds tested, results of in vitro and in vivo studies provide substantial evidence for mutagenic activity of hexavalent chromium compounds. The mutagenicity of hexavalent chromium is mediated through the generation of highly reactive chromium intermediates (e.g., chromium(IV) and chromium(V)) and reactive oxygen species formed during the intracellular reduction of hexavalent chromium. Reactive chromium intermediates and oxygen species react with DNA, leading to oxidative DNA damage, chromium-DNA adducts, DNA strand breaks, and chromosomal aberrations.

In in vitro test systems, hexavalent chromium compounds have mostly tested positive for gene mutations (including reverse mutations, frame shift mutations, and base pair substitutions) and DNA damage (including DNA-protein crosslinks) in bacterial cells (*S. typhimurium, E. coli, B. subtilis*); for forward mutations and mitotic gene conversion in yeast (*S. cerevisiae*); and for DNA damage (DNA strand breaks, fragmentation, DNA-protein crosslinks, DNA-DNA crosslinks), chromosomal damage (sister chromatid exchanges and chromosomal aberrations), and DNA synthesis inhibition in mammalian cell lines and primary cell cultures (including

primary cell cultures of human gastric mucosal cells, respiratory tract cells, and lymphocytes). In in vivo test systems, hexavalent chromium compounds have tested positive for mutations in *D. melanogaster* and for DNA damage (DNA-protein crosslinks, DNA strand breaks), mutations, chromosomal damage (sister chromatid exchanges, chromosomal aberrations, and micronuclei), and DNA synthesis inhibition in rats and mice. Thus, the mutagenic activity of hexavalent chromium has been demonstrated in numerous studies using both in vitro and in vivo experimental systems.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), hexavalent chromium is "likely to be carcinogenic to humans" via the oral route of exposure based on a statistically significant increase in the incidence of tumors of the oral mucosa and tongue of rats and of the small intestine of mice, and evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans. Additionally, available evidence indicates that chromium interacts with DNA, resulting in DNA damage and mutagenesis. Based on the weight of the available evidence, hexavalent chromium is proposed to act through a mutagenic mode of carcinogenic action, and thus, ADAFs should be applied.

6.2. DOSE RESPONSE

6.2.1. Noncancer—Oral

NTP (2008), a 2-year animal bioassay that used multiple dose groups and included a comprehensive assessment of endpoints, was selected as the principal study for derivation of the RfD. Dose-response analysis using BMD methods was conducted for the following endpoints from this study: histopathological changes in the liver (chronic inflammation in female rats and histiocytic cellular infiltration in female mice), duodenum (diffuse epithelial hyperplasia in male and female mice), mesenteric lymph node (histiocytic cellular infiltration in male and female mice), and pancreas (cytoplasm cellular alteration of acinar epithelial cells in female mice).

All available dichotomous models in EPA's BMDS were fit to the incidence data for the selected endpoints, using 10% extra risk as the BMR in accordance with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b).

Based on the lowest $BMDL_{10}$ value of 0.09 mg hexavalent chromium/kg-day, diffuse epithelial hyperplasia of the duodenum in female mice was selected as the POD for derivation of the RfD. The RfD of 0.0009 or 9×10^{-4} mg/kg-day for hexavalent chromium was derived by dividing the $BMDL_{10}$ (or POD) of 0.09 mg/kg-day by a composite uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Confidence in the principal study, NTP (2008), is high. NTP (2008) is a 2-year toxicology and carcinogenicity study of sodium dichromate dihydrate in drinking water in rats and mice that used a relevant route of exposure (drinking water) and a robust study design (i.e., 50 animals/sex/group, a control and four exposed groups, measurements of drinking water consumption, and a full suite of hematology, clinical chemistry, and gross and miscroscopic

tissue examinations). Confidence in the oral database is medium to high. The database includes subchronic and chronic drinking water studies in rats and mice conducted by NTP (2008, 2007), a second chronic drinking water study in rats, and several other subchronic oral toxicity studies in rats and mice. Other than the NTP bioassays, however, the available subchronic and chronic toxicity studies did not provide adequate characterization of both the NOAEL and LOAEL for the hexavalent chromium compounds tested. Studies have been conducted to evaluate the effects of ingested hexavalent chromium compounds on reproductive tissues, mating behavior, reproductive outcomes, and fetal development. The reproductive toxicity database includes a continuous breeding study by NTP. Overall confidence in the RfD is medium to high.

6.2.2. Cancer—Oral

The mode of action is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with hexavalent chromium exposures. This approach is supported by the evidence for genotoxicity and a mutagenic mode of action.

The CSF for hexavalent chromium is based on tumor incidence data from the NTP (2008) animal bioassay. The incidence of neoplasms in the small intestine of mice was used to derive the CSF. Only animals that survived for at least 451 days, the time until appearance of the first tumor, were considered at risk for tumor development.

BMD modeling was carried out using the multistage model in EPA's BMDS (U.S. EPA, 2000b) to identify a POD. In applying the BMD approach to the derivation of a CSF, the lower 95% confidence bound on the dose corresponding to the BMR (defined as 10% extra risk of small intestine tumors) was calculated. This lower confidence bound is referred to as the BMDL. The CSF was calculated by dividing the BMR by the BMDL, and then converting this CSF to human equivalents using body weight to the ¾ power scaling.

The CSF resulting from the fitting of the multistage model in BMDS to the incidence of neoplasms in the small intestine of male and female mice was 0.5 $(mg/kg\text{-}day)^{-1}$ and 0.6 $(mg/kg\text{-}day)^{-1}$, respectively, expressed in human equivalents. Because of the poorer fit of the multistage model to the female mouse data, the cancer potency estimate of 0.5 $(mg/kg\text{-}day)^{-1}$ based on the male mouse data was selected as the CSF for hexavalent chromium.

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

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APPENDIX B. BENCHMARK DOSE CALCULATIONS

B.1. DETAILS OF BMD ANALYSIS FOR THE RFD

Table B-1. Incidence data for nonneoplastic lesions from all treatment groups of female F344/N rats and male and female B6C3F$_1$ mice exposed to sodium dichromate dihydrate in drinking water for 2 years (NTP, 2008)

	Dose (mg hexavalent chromium/kg-d)				
	0	0.24	0.94	2.4	7.0
Female rats					
Liver: chronic inflammation	12/50	21/50[a]	28/50[b]	35/50[b]	39/50[b]
	Dose (mg hexavalent chromium/kg-d)				
	0	0.38	0.91	2.4	5.9
Male mice					
Duodenum: diffuse epithelial hyperplasia	0/50	11/50[b]	18/50[b]	42/50[b]	32/50[a]
Mesenteric lymph node: histiocytic cellular infiltration	14/47	38/47[b]	31/49[b]	32/49[b]	42/46[a]
	Dose (mg hexavalent chromium/kg-d)				
	0	0.38	1.4	3.1	8.7
Female mice					
Duodenum: diffuse epithelial hyperplasia	0/50	16/50[b]	35/50[b]	31/50[b]	42/50[b]
Mesenteric lymph node: histiocytic cellular infiltration	3/46	29/48[b]	26/46[b]	40/50[b]	42/50[b]
Liver: histiocytic cellular infiltration	2/49	15/50[b]	23/50[b]	32/50[b]	45/50[b]
Pancreas: acinus, cytoplasmic alteration	0/48	6/50[a]	6/49[a]	14/50[b]	32/50[b]

[a]Significantly different ($p \leq 0.05$) from the control group by Dunn's or Shirley's test.
[b]Significantly different ($p \leq 0.01$) from the control group by Dunn's or Shirley's test.

Source: ATSDR (2008).

Chronic inflammation of the liver in female rats. As assessed by the χ^2 goodness-of-fit statistic, only the log-logistic model provided an adequate fit (χ^2 p-value ≥ 0.1) to the data (Table B-2). Based on the log-logistic model, the BMD associated with a 10% extra risk was 0.22 mg hexavalent chromium/kg-day and its lower 95% confidence limit (BMDL) was 0.14 mg hexavalent chromium/kg-day (Figure B-1).

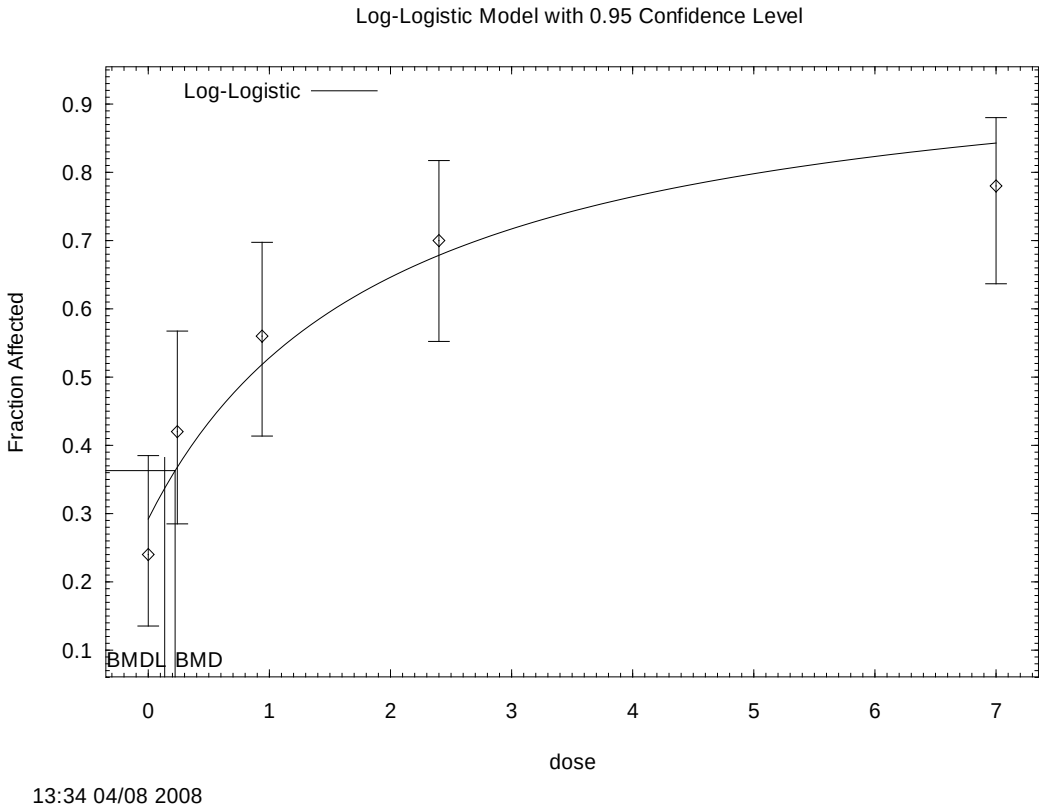
Table B-2. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for chronic inflammation of the liver in female rats exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
Gamma[a]	0.51	0.37	0.04	317.97
Logistic	0.84	0.65	0.01	321.45
Log-logistic[b]	**0.22**	**0.14**	**0.37**	**312.57**
Multistage[c]	0.51	0.37	0.04	317.97
Probit	0.88	0.70	0.01	321.80
Log-probit[b]	0.89	0.61	0.01	320.86
Quantal linear	0.51	0.37	0.04	317.97
Weibull[a]	0.51	0.37	0.04	317.97

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 3-degree polynomial is reported.

AIC = Akaike's information criterion

Source: ATSDR (2008).



BMDs and BMDLs indicated are associated with a 10% extra risk, and are in units of mg hexavalent chromium/kg-day.

Source: ATSDR (2008).

Figure B-1. Predicted and observed incidence of chronic inflammation of the liver in female rats exposed to sodium dichromium dihydrate in drinking water for 2 years.

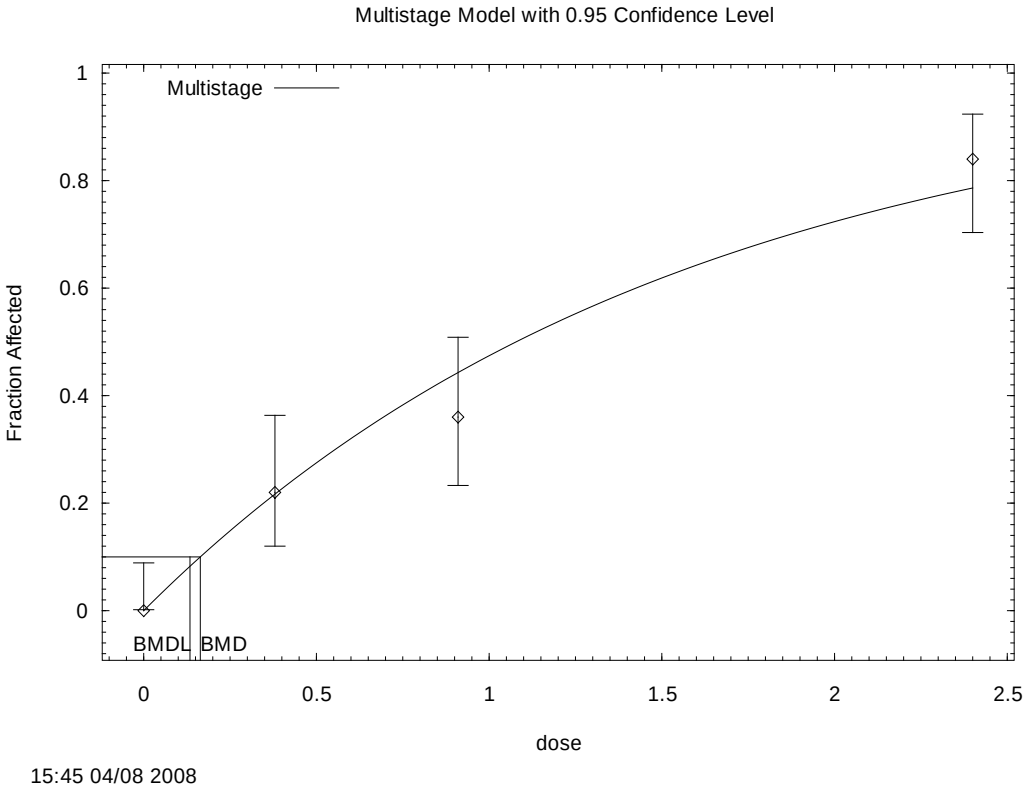
Diffuse epithelial hyperplasia of the duodenum in male mice. As assessed by the χ^2 goodness-of-fit statistic, none of the models provided an adequate fit (χ^2 *p*-value ≥ 0.1) to the full dataset (Table B-3). In order to achieve an adequately fitting model, the highest dose was dropped. This is determined to be appropriate, as the area of concern is with the low-dose region of the response curve. After dropping the highest dose, the gamma, log-logistic, multistage, log-probit, quantal linear, and Weibull models provided adequate fits to the data (χ^2 *p*-value > 0.1). Comparing across models, a better fit is generally indicated by a lower Akaike's Information Criterion (AIC) (EPA, 2000b). As assessed by AIC, the 1-degree polynomial multistage model provided the best fit to the data (Figure B-2). Based on the multistage model, the BMD associated with a 10% extra risk was 0.16 mg hexavalent chromium/kg-day and its lower 95% confidence limit (BMDL) was 0.13 mg hexavalent chromium/kg-day.

Table B-3. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for diffuse epithelial hyperplasia in the duodenum in male mice exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
All doses				
Gamma[a]	0.31	0.25	0.00	270.99
Logistic	0.90	0.74	0.00	296.25
Log-logistic[b]	0.15	0.12	0.00	247.93
Multistage[c]	0.31	0.25	0.00	270.99
Probit	0.90	0.76	0.00	296.18
Log-probit[b]	0.48	0.36	0.00	274.38
Quantal linear	0.31	0.25	0.00	270.99
Weibull[a]	0.31	0.25	0.00	270.99
Highest dose dropped (four doses modeled)				
Gamma[a]	0.22	0.14	0.43	167.67
Logistic	0.47	0.39	0.03	177.09
Log-logistic[b]	0.26	0.15	0.20	169.23
Multistage[d]	0.16	**0.13**	**0.52**	**166.34**
Probit	0.45	0.37	0.04	176.19
Log-probit[b]	0.28	0.23	0.33	167.41
Quantal linear	0.16	**0.13**	**0.52**	**166.34**
Weibull[a]	0.22	0.14	0.47	167.50

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 3-degree polynomial is reported.
[d]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; degree polynomial = 1.

Source: ATSDR (2008).



BMDs and BMDLs indicated are associated with a 10% extra risk, and are in units of mg hexavalent chromium/kg-day.

Source: ATSDR (2008).

Figure B-2. Predicted and observed incidence of diffuse epithelial hyperplasia in the duodenum of male mice exposed to sodium dichromium dihydrate in drinking water for 2 years.

Histiocytic cellular infiltration of the mesenteric lymph nodes in male mice. As assessed by the χ^2 goodness-of-fit statistic, none of the models provided an adequate fit (χ^2 *p*-value ≥ 0.1) to the full dataset (Table B-4). In order to achieve a statistically fit model, the highest dose was dropped. This is determined to be appropriate, as the area of concern is with the low-dose region of the response curve. Dropping the highest dose did not result in adequately fitting models, nor did dropping the two highest doses. This dataset is considered not suitable for BMD modeling.

Table B-4. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for histiocytic cellular infiltration in mesenteric lymph nodes of male mice exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
All doses				
Gamma[a]	0.38	0.26	0.00	285.94
Logistic	0.53	0.39	0.00	286.38
Log-logistic[b]	0.16	0.08	0.00	284.48
Multistage[c]	0.43	0.26	0.00	287.88
Probit	0.56	0.43	0.00	286.35
Log-probit[b]	0.83	0.52	0.00	289.36
Quantal linear	0.38	0.26	0.00	285.94
Weibull[a]	0.38	0.26	0.00	285.94
Highest dose dropped (four doses modeled)				
Gamma[a]	0.47	0.24	0.00	258.50
Logistic	0.61	0.35	0.00	259.04
Log-logistic[b]	0.21	0.08	0.00	256.81
Multistage[d]	0.47	0.24	0.00	258.50
Probit	0.63	0.37	0.00	259.08
Log-probit[b]	1.24	0.56	0.00	261.28
Quantal linear	0.47	0.24	0.00	258.50
Weibull[a]	0.47	0.24	0.00	258.50
Two highest doses dropped (three doses modeled)				
Gamma[a]	0.11	0.07	0.00	187.77
Logistic	0.17	0.12	0.00	189.97
Log-logistic[b]	0.05	0.03	0.00	183.77
Multistage[e]	0.11	0.07	0.00	187.77
Probit	0.17	0.12	0.00	190.12
Log-probit[b]	0.17	0.11	0.00	190.37
Quantal linear	0.11	0.07	0.00	187.77
Weibull[a]	0.11	0.07	0.00	187.77

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 3-degree polynomial is reported.
[d]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 2-degree polynomial is reported.
[e]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 1-degree polynomial is reported.

Source: ATSDR (2008).

Diffuse epithelial hyperplasia of the duodenum in female mice. As assessed by the χ^2 goodness-of-fit statistic, none of the models provided an adequate fit (χ^2 *p*-value ≥ 0.1) to the

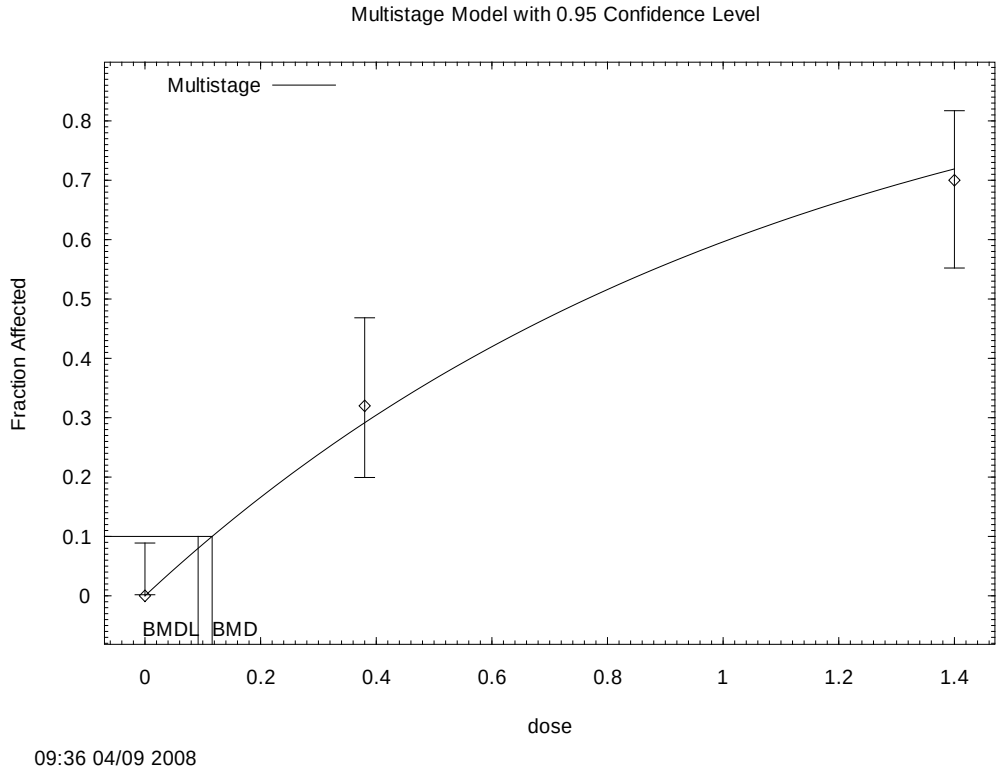
data (Table B-5). In order to achieve a statistically fit model, the highest dose was dropped. This is determined to be appropriate, as the area of concern is with the low-dose region of the response curve. After dropping the highest dose, an adequate fit was still not achieved. After dropping the two highest doses, all of the models except for the logistic and probit models provided an adequate fit (χ^2 *p*-value ≥ 0.1) to the data. Comparing across models, a better fit is generally indicated by a lower AIC (EPA, 2000b). As assessed by AIC, the gamma, multistage, quantal linear, and Weibull models generated identical goodness of fit statistics and BMD, as these models all took the form of a 1-degree polynomial multistage model, which provides the best fit (Figure B-3). Based on these models, the BMD associated with a 10% extra risk was 0.12 mg hexavalent chromium/kg-day and its lower 95% confidence limit (BMDL) was 0.09 mg hexavalent chromium/kg-day.

Table B-5. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for diffuse epithelial hyperplasia in the duodenum of female mice exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
All doses				
Gamma[a]	0.34	0.27	0.00	275.34
Logistic	0.88	0.72	0.00	293.17
Log-logistic[b]	0.12	0.09	0.04	245.54
Multistage[c]	0.34	0.27	0.00	275.34
Probit	0.93	0.78	0.00	294.03
Log-probit[b]	0.52	0.38	0.00	279.54
Quantal linear	0.34	0.27	0.00	275.34
Weibull[a]	0.34	0.27	0.00	275.34
Highest dose dropped (four doses modeled)				
Gamma[a]	0.20	0.16	0.00	213.41
Logistic	0.55	0.46	0.00	236.10
Log-logistic[b]	0.11	0.08	0.04	200.07
Multistage[d]	0.20	0.16	0.00	213.41
Probit	0.54	0.45	0.00	235.61
Log-probit[b]	0.29	0.24	0.00	220.04
Quantal linear	0.20	0.16	0.00	213.41
Weibull[a]	0.20	0.16	0.00	213.41
Two highest doses dropped (three doses modeled)				
Gamma[a]	**0.12**	**0.09**	**0.87**	**126.06**
Logistic	0.34	0.27	0.00	141.77
Log-logistic[b]	0.12	0.06	1.00	127.77
Multistage[e]	**0.12**	**0.09**	**0.87**	**126.06**
Probit	0.32	0.26	0.00	140.65
Log-probit[b]	0.20	0.16	0.48	127.17
Quantal linear	**0.12**	**0.09**	**0.87**	**126.06**
Weibull[a]	**0.12**	**0.09**	**0.87**	**126.06**

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 3-degree polynomial is reported.
[d]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 2-degree polynomial is reported.
[e]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 1-degree polynomial is reported.

Source: ATSDR (2008).



BMDs and BMDLs indicated are associated with a 10% extra risk, and are in units of mg hexavalent chromium/kg-day.

Source: ATSDR (2008).

Figure B-3. Predicted and observed incidence of diffuse epithelial hyperplasia in the duodenum of female mice exposed to sodium dichromium dihydrate in drinking water for 2 years.

Histiocytic cellular infiltration of the mesenteric lymph nodes in female mice. As assessed by the χ^2 goodness-of-fit statistic, none of the models provided an adequate fit (χ^2 *p*-value ≥ 0.1) to the full dataset (Table B-6). In order to achieve a statistically fit model, the highest dose was dropped. This is determined to be appropriate, as the area of concern is with the low-dose region of the response curve. Dropping the highest dose did not result in adequately fitting models, nor did dropping the two highest doses. This dataset is not suitable for BMD modeling.

Table B-6. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for histiocytic cellular infiltration in mesenteric lymph nodes of female mice exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
All doses				
Gamma[a]	0.41	0.30	0.00	282.46
Logistic	0.77	0.61	0.00	290.18
Log-logistic[b]	0.09	0.06	0.00	263.55
Multistage[c]	0.41	0.30	0.00	282.46
Probit	0.85	0.69	0.00	291.41
Log-probit[b]	0.68	0.47	0.00	285.85
Quantal linear	0.41	0.30	0.00	282.46
Weibull[a]	0.41	0.30	0.00	282.46
Highest dose dropped (four doses modeled)				
Gamma[a]	0.20	0.15	0.00	224.84
Logistic	0.40	0.33	0.00	230.81
Log-logistic[b]	0.07	0.05	0.00	215.19
Multistage[d]	0.20	0.15	0.00	224.84
Probit	0.40	0.34	0.00	230.85
Log-probit[b]	0.37	0.24	0.00	231.76
Quantal linear	0.20	0.15	0.00	224.84
Weibull[a]	0.20	0.15	0.00	224.84
Two highest doses dropped (three doses modeled)				
Gamma[a]	0.14	0.10	0.00	172.32
Logistic	0.31	0.24	0.00	178.99
Log-logistic[b]	0.07	0.04	0.00	164.47
Multistage[e]	0.14	0.10	0.00	172.32
Probit	0.30	0.23	0.00	178.74
Log-probit[b]	0.21	0.15	0.00	178.11
Quantal linear	0.14	0.10	0.00	172.32
Weibull[a]	0.14	0.10	0.00	172.32

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 3-degree polynomial is reported.
[d]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 2-degree polynomial is reported.
[e]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 1-degree polynomial is reported.

Source: ATSDR (2008).

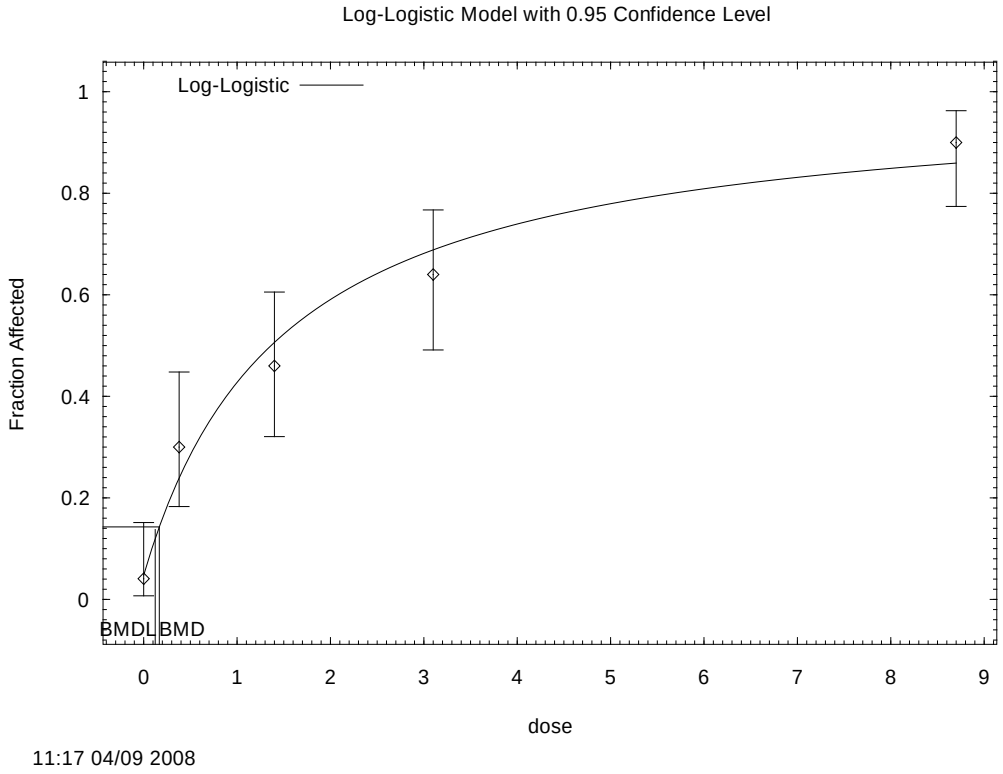
Histiocytic cellular infiltration of the liver in female mice. As assessed by the χ^2 goodness-of-fit statistic, only the log-logistic model provided an adequate fit (χ^2 *p*-value ≥ 0.1) to the data (Table B-7). Based on the log-logistic model, the BMD associated with a 10% extra risk was 0.17 mg hexavalent chromium/kg-day and its lower 95% confidence limit (BMDL) was 0.12 mg hexavalent chromium/kg-day (Figure B-4).

Table B-7. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for histiocytic cellular infiltration in the liver of female rats exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
Gamma[a]	0.35	0.28	0.08	255.40
Logistic	0.85	0.70	0.00	267.56
Log-logistic[b]	**0.17**	**0.12**	**0.44**	**251.36**
Multistage[c]	0.35	0.28	0.08	255.40
Probit	0.88	0.75	0.00	268.64
Log-probit[b]	0.62	0.48	0.01	260.00
Quantal linear	0.35	0.28	0.08	255.40
Weibull[a]	0.35	0.28	0.08	255.40

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; no degree of polynomial provided a fit, a 3-degree polynomial is reported.

Source: ATSDR (2008).



BMDs and BMDLs indicated are associated with a 10% extra risk, and are in units of mg hexavalent chromium/kg-day.

Source: ATSDR (2008).

Figure B-4. Predicted and observed incidence of histiocytic cellular infiltration in the livers of female mice exposed to sodium dichromium dihydrate in drinking water for 2 years.

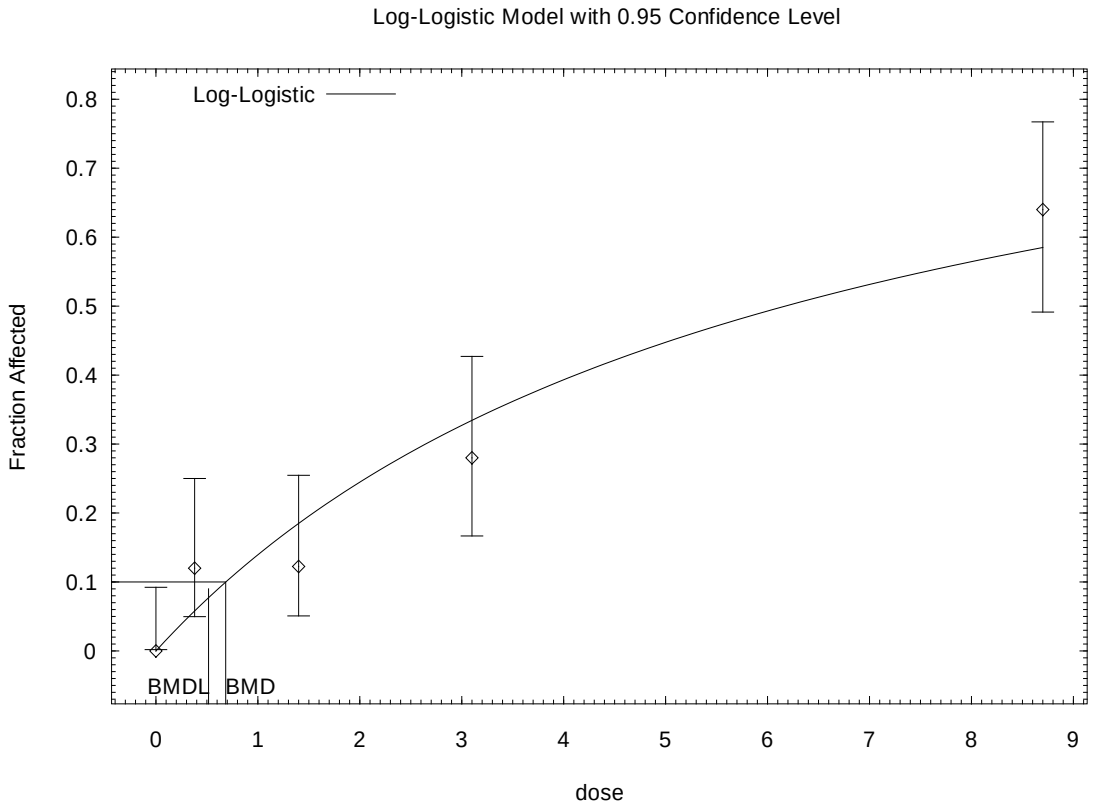
Cytoplasmic alteration of acinar epithelial cells of the pancreas in female mice. As assessed by the χ^2 goodness-of-fit statistic, all of the models provide adequate fits (χ^2 *p*-value ≥ 0.1) to the data (Table B-8). Comparing across models, a better fit is generally indicated by a lower AIC (EPA, 2000b). As assessed by AIC, the log-logistic model provides the best fit (Figure B-5). Based on the log-logistic model, the BMD associated with a 10% extra risk was 0.68 mg hexavalent chromium/kg-day and its lower 95% confidence limit (BMDL) was 0.52 mg hexavalent chromium/kg-day.

Table B-8. BMD_{10} and $BMDL_{10}$ values and goodness-of-fit statistics from models fit to incidence data for pancreas: acinus, cytoplasmic alteration in female mice exposed to sodium dichromium dihydrate in drinking water for 2 years

Model	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	χ^2 *p*-value	AIC
Gamma[a]	0.92	0.72	0.13	206.82
Logistic	2.43	2.03	0.09	211.78
Log-logistic[b]	**0.68**	**0.52**	**0.19**	**205.22**
Multistage[c]	0.92	0.72	0.13	206.82
Probit	2.24	1.89	0.11	210.99
Log-probit[b]	1.77	1.40	0.11	209.99
Quantal linear	0.92	0.72	0.13	206.82
Weibull[a]	0.92	0.72	0.13	206.82

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial (up to n-2) with an adequate fit is reported; a 1-degree polynomial is reported.

Source: ATSDR (2008).



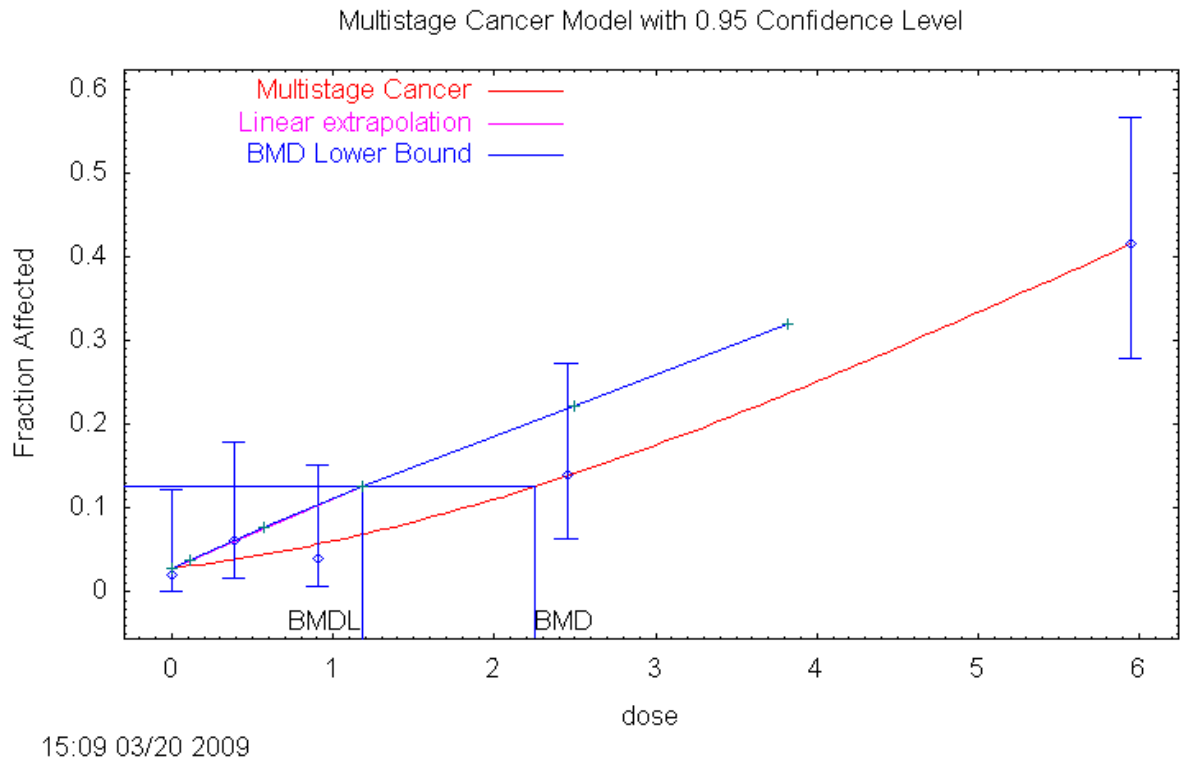
BMDs and BMDLs indicated are associated with a 10% extra risk, and are in units of mg hexavalent chromium/kg-day.

Source: ATSDR (2008).

Figure B-5. Predicted and observed incidence of pancreas: acinus, cytoplasmic alteration in female mice exposed to sodium dichromium dihydrate in drinking water for 2 years.

B.2. DETAILS OF BMD ANALYSIS FOR THE ORAL SLOPE FACTOR

The fit of the multistage model to the incidence of neoplasms in the small intestine of male mice administered sodium dichromate dihydrate in drinking water for 2 years (NTP, 2008):



Source: NJDEP (2009)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File:
M:\ChromiumVI\msc_MALE_MICE_INTESTINAL_TUMORS_NTP_2008_Setting.(d)
        Gnuplot Plotting File:
M:\ChromiumVI\msc_MALE_MICE_INTESTINAL_TUMORS_NTP_2008_Setting.plt
                                          Fri Feb 05 09:42:31 2010
 ======================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive
```

Dependent variable = Response
Independent variable = Dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0291151
Beta(1) = 0.0232273
Beta(2) = 0.0107072

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	-0.73	0.62
Beta(1)	-0.73	1	-0.96
Beta(2)	0.62	-0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0287353	*	*	*
Beta(1)	0.024191	*	*	*
Beta(2)	0.0105146	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-77.3728	5			
Fitted model	-77.8649	3	0.984149	2	0.6114
Reduced model	-96.8272	1	38.9088	4	<.0001

AIC: 161.73

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0287	1.408	1.000	49	-0.349
0.3800	0.0391	1.915	3.000	49	0.800
0.9100	0.0581	2.848	2.000	49	-0.518
2.4000	0.1374	6.869	7.000	50	0.054
5.9000	0.4160	19.969	20.000	48	0.009

Chi^2 = 1.03 d.f. = 2 P-value = 0.5968

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.21769

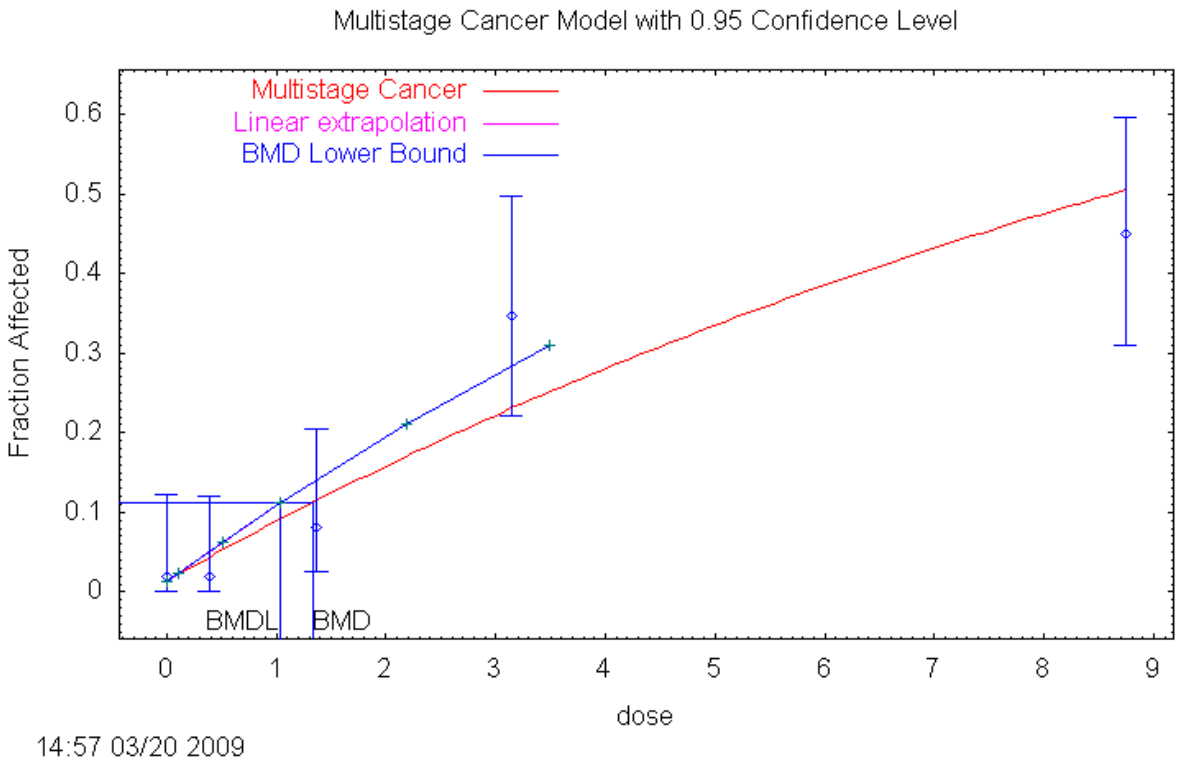
BMDL = 1.16524

BMDU = 3.23024

Taken together, (1.16524, 3.23024) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.085819

The fit of the multistage model to the incidence of neoplasms in the small intestine of female mice administered sodium dichromate dihydrate in drinking water for 2 years (NTP, 2008):



Source: NJDEP (2009)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File:
M:\ChromiumVI\msc_FEMALE_MICE_INTESTINAL_TUMORS_NTP_2008_Setting.(d)
        Gnuplot Plotting File:
M:\ChromiumVI\msc_FEMALE_MICE_INTESTINAL_TUMORS_NTP_2008_Setting.plt
                                          Fri Feb 05 09:54:51 2010
 ======================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose
```

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0398439
Beta(1) = 0.0695693
Beta(2) = 0

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)
Background	1	-0.62
Beta(1)	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0140838	*	*	*
Beta(1)	0.0792034	*	*	*
Beta(2)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-88.9774	5			
Fitted model	-91.8504	2	5.74595	3	0.1246
Reduced model	-117.047	1	56.1401	4	<.0001

AIC: 187.701

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0141	0.690	1.000	49	0.376
0.3800	0.0433	2.166	1.000	50	-0.810
1.4000	0.1176	5.761	4.000	49	-0.781
3.1000	0.2287	11.208	17.000	49	1.970
8.7000	0.5050	24.746	22.000	49	-0.785

Chi^2 = 5.90 d.f. = 3 P-value = 0.1164

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.33025

BMDL = 1.02757

BMDU = 1.93668

Taken together, (1.02757, 1.93668) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0973173