3. HEALTH EFFECTS

3.1 INTRODUCTION

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

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

Chromium is a naturally occurring element found in animals, plants, rocks, and soil and in volcanic dust and gases. Chromium has oxidation states (or "valence states") ranging from chromium(-II) to chromium(VI). Elemental chromium (chromium(0)) does not occur naturally. Chromium compounds are stable in the trivalent state and occur in nature in this state in ores, such as ferrochromite. The hexavalent (VI) form is the second-most stable state. However, chromium(VI) rarely occurs naturally, but is usually produced from anthropogenic sources (EPA 1984a).

Trivalent chromium compounds, except for acetate, nitrate, and chromium(III) chloride-hexahydrate salts, are generally insoluble in water. Some hexavalent compounds, such as chromium trioxide (or chromic acid) and the ammonium and alkali metal (e.g., sodium, potassium) salts of chromic acid are readily soluble in water. The alkaline metal (e.g., calcium, strontium) salts of chromic acid are less soluble in water. The zinc and lead salts of chromic acid are practically insoluble in cold water. Chromium(VI) compounds are reduced to chromium(III) in the presence of oxidizable organic matter. However, in natural waters where there is a low concentration of reducing materials, chromium(VI) compounds are more stable (EPA 1984a). For more information on the physical and chemical properties of chromium, see Chapter 4.

In humans and animals, chromium(III) is an essential nutrient that plays a role in glucose, fat, and protein metabolism by potentiating the action of insulin (Anderson 1981). The biologically active form of chromium, called chromodulin, is an oligopeptide complex containing with four chromic ions (Jacquamet et al. 2003). Both humans and animals are capable of converting inactive inorganic chromium(III) compounds to physiologically active forms. The nutritional role of chromium is further discussed in Section 3.4.3. Although chromium(III) has been reported to be an essential nutrient, exposure to high

levels via inhalation, ingestion, or dermal contact may cause some adverse health effects. Most of the studies on health effects discussed below involve exposure to chromium(III) and chromium(VI) compounds. In addition, chromium(IV) was used in an inhalation study to determine permissible exposure levels for workers involved in producing magnetic tape (Lee et al. 1989).

Several factors should be considered when evaluating the toxicity of chromium compounds. The purity and grade of the reagent used in the testing is an important factor. Both industrial- and reagent-grade chromium(III) compounds can be contaminated with small amounts of chromium(VI) (Levis and Majone 1979). Thus, interpretation of occupational and animal studies that involve exposure to chromium(III) compounds is difficult when the purity of the compounds is not known. In addition, it is difficult to distinguish between the effects caused by chromium(VI) and those caused by chromium(III) since chromium(VI) is rapidly reduced to chromium(III) after penetration of biological membranes and in the gastric environment (Petrilli et al. 1986b; Samitz 1970). However, whereas chromium(VI) can readily be transported into cells, chromium(III) is much less able to cross cell membranes. The reduction of chromium(VI) to chromium(III) inside of cells may be an important mechanism for the toxicity of chromium compounds, whereas the reduction of chromium(VI) to chromium(III) outside of cells is a major mechanism of protection.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be

classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) are indicated in Tables 3-1 and 3-3 and Figures 3-1 and 3-3 for chromium(VI).

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

3.2.1 Inhalation Exposure

Due to the extremely high boiling point of chromium, gaseous chromium is rarely encountered. Rather, chromium in the environment occurs as particle-bound chromium or chromium dissolved in droplets. As discussed in this section, chromium(VI) trioxide (chromic acid) and soluble chromium(VI) salt aerosols may produce different health effects than insoluble particulate compounds. For example, exposure to chromium(VI) trioxide results in marked damage to the nasal mucosa and perforation of the nasal septum, whereas exposure to insoluble(VI) compounds results in damage to the lower respiratory tract.

3.2.1.1 Death

No studies were located regarding death in humans after acute inhalation of chromium or chromium compounds. An increased risk of death from noncancer respiratory disease was reported in retrospective mortality studies of workers in a chrome plating plant (Sorahan et al. 1987) and chromate production (Davies et al. 1991; Taylor 1966) (see Section 3.2.1.2, Respiratory Effects). However, a number of methodological deficiencies in these studies prevent the establishment of a definitive cause-effect relationship. Retrospective mortality studies associating chromium exposure with cancer are discussed in Section 3.2.1.7.

Acute inhalation LC_{50} values in rats for several chromium(VI) compounds (sodium chromate, sodium dichromate, potassium dichromate, and ammonium dichromate) ranged from 29 to 45 mg chromium(VI)/m^3 for females and from 33 to 82 mg chromium(VI)/m^3 for males (Gad et al. 1986). Acute inhalation LC_{50} values for chromium trioxide were 87 and 137 mg chromium(VI)/m^3 for female and male rats, respectively (American Chrome and Chemicals 1989). Female rats were more sensitive than males to the lethal effects of most chromium(VI) compounds except sodium chromate, which was equally toxic in both sexes. Signs of toxicity included respiratory distress, irritation, and body weight depression (Gad et al. 1986). The LC_{50} values for chromium(VI) are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal effects in humans or animals after inhalation exposure to chromium or its compounds. Respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, dermal, ocular, and body weight effects are discussed below. The highest NOAEL values and all reliable LOAEL values for each systemic effect in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1 for chromium(VI) and recorded in Table 3-2 and plotted in Figure 3-2 for chromium(III).

Respiratory Effects. The respiratory tract in humans is a major target of inhalation exposure to chromium compounds. Chromate sensitive workers acutely exposed to chromium(VI) compounds may develop asthma and other signs of respiratory distress. Five individuals who had a history of contact dermatitis to chromium were exposed via a nebulizer to an aerosol containing 0.035 mg chromium(VI)/mL as potassium dichromate. A 20% decrease in the forced expiratory volume of the lungs was observed and was accompanied by erythema of the face, nasopharyngeal pruritus, nasal blocking, coughing, and wheezing (Olaguibel and Basomba 1989).

Table 3-1 Levels of Significant Exposure to Chromium VI - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Fischer- 344)	4 hr				137 M (LC50) 87 F (LC50)	American Chrome and Chemicals 1989 CrO3 (VI)	
2	Rat (Fischer- 344)	4 hr				82 M (LC50) 45 F (LC50)	Gad et al. 1986 (NH4)2Cr2O7 (VI)	
3	Rat (Fischer- 344)	4 hr				35 M (LC50) 29 F (LC50)	Gad et al. 1986 K2Cr2O7 (VI)	
4	Rat (Fischer- 344)	4 hr				70 M (LC50) 31 F (LC50)	Gad et al. 1986 Na2Cr2O7.2H2O (VI)	
5	Rat (Fischer- 344)	4 hr				33 (LC50)	Gad et al. 1986 Na2CrO4 (VI)	
Systemic								
6	Rat (Sprague-Dawley)	10 d 5 d/wk 6 hr/d	Resp	0.49 M	1.15 M (nasal hemorrhage)		Kim et al. 2004 CrO3 (VI)	
INTERMEDIATE EXPOSURE								
Systemic								
7	Human	<90 d (occup)	Resp		0.025 M (irritated nasal septum)		Gibb et al. 2000a CrO3 (VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
8	Human	90 d- 1 yr (occup)	Resp		0.033 M perforated nasal septum		Gibb et al. 2000a CrO3 (VI)	
			Other		0.036 M perforated eardrum			
9	Human	<1 yr 5 d/wk 8 hr/d (occup)	Resp		0.1 (epitaxis rhinorrhea, nasal ulceration and perforation)		Gomes 1972 CrO3 (VI)	
10	Human	0.5-12 mo 6 mo avg 5 d/wk 8 hr/d (occup)	Resp		0.09 M (epitaxis, rhinorrhea ulceration of nasal septum)		Kleinfield and Rosso 1965 CrO3 (VI)	
11	Human	0.2-23.6 yr avg 2.5 yr 5 d/wk 8 hr/d (occup)	Resp		0.002[b] (nasal mucosa atrophy and ulceration, mild decreased lung function)		Lindberg and Hedenstierna 1983 CrO3 (VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
12	Rat (Wistar)	28 d 7 d/wk 22 hr/d	Resp		0.025 M (increased percentage of lymphocytes in bronchoalveolar lavage fluid)		Glaser et al. 1985 Na2Cr2O7.2H2O (VI)	
			Gastro	0.2 M				
			Hemato	0.2 M				
			Hepatic	0.2 M				
			Renal	0.2 M				
			Bd Wt	0.2 M				
13	Rat (Wistar)	90 d 7 d/wk 22 hr/d	Resp		0.025 M (increased percentage of lymphocytes in bronchial alveolar lavage fluid)		Glaser et al. 1985 Na2Cr2O7.2H2O (VI)	
			Gastro	0.2 M				
			Hemato	0.2 M				
			Hepatic	0.1 M	0.2 M (increased levels of serum phospholipids and triglycerides)			
			Renal	0.2 M				
			Bd Wt	0.2 M				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
14	Rat (Wistar)	30 or 90 d 7 d/wk 22 hr/d	Resp		0.05 M[c] (increased lung weight, hyperplasia, macrophage infiltration, increased protein, albumin, lactate dehydrogenase in BAL fluid)		Glaser et al. 1990 Na2Cr2O7.2H2O (VI)	
			Gastro	0.4 M				
			Hemato		0.05 M (increased white blood cell count)			
			Hepatic	0.4 M				
			Renal	0.4 M				
			Bd Wt	0.1 M	0.2 M (28% decreased body weight gain)			
15	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Resp	0.23 M	0.49 M (inflammation and macrophage aggregation in alveolar regions of the lung)		Kim et al. 2004 CrO3 (VI)	
			Cardio	1.15 M				
			Hemato		0.23 M (decreased hematocrit)			
			Hepatic	1.15 M				
			Renal	1.15 M				
			Endocr	1.15 M				
			Bd Wt	1.15 M				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
16	Mouse (C57BL)	12 mo 2 d/wk 120 min/d	Resp			1.81 F (emphysema, nasal septum perforation)	Adachi 1987 CrO3 (VI)	
17	Mouse (ICR)	12 mo 2 d/wk 30 min/d	Resp			3.63 F (emphysema, nasal septum perforation)	Adachi et al. 1986 CrO3 (VI)	
18	Rabbit (NS)	4-6 wk 5 d/wk 6 hr/d	Resp	0.9 M			Johansson et al. 1986b Na2CrO4 (VI)	
Immuno/ Lymphoret								
19	Rat (Fischer- 344)	2-4 wk 5 d/wk 5 hr/d			0.36 (increased neutrophils, monocytes, and decreased macrophages in BAL fluid; decreased cytokine levels)		Cohen et al. 1998 K2CrO4 (VI)	
20	Rat (Fischer- 344)	2-4 wk 5 d/wk 5 hr/d			0.36 (decreased tumor necrosis factor-alpha levels and production of superoxide anion and hydrogen peroxide and increased nitric oxide production)		Cohen et al. 1998 BaCrO4 (VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
21	Rat (Wistar)	28 d 7 d/wk 22 hr/d			0.025 M (increased response to sheep red blood cells, increased percentage of lymphocytes in bronchoalveolar lavage fluid)		Glaser et al. 1985 Na2Cr2O7.2H2O (VI)	
22	Rat (Wistar)	90 d 7 d/wk 22 hr/d			0.025 M (increased response to sheep RBC, increased % of lymphocytes in bronchoalveolar lavage fluid, increased % of macrophages in telophase, increased activity of macrophages)		Glaser et al. 1985 Na2Cr2O7.2H2O (VI)	
Neurological								
23	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1.15 M			Kim et al. 2004 CrO3 (VI)	
Reproductive								
24	Rat (Wistar)	90 d 7 d/wk 22 hr/d		0.2 M			Glaser et al. 1985 Na2Cr2O7.2H2O (VI)	
25	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1.15 M			Kim et al. 2004 CrO3 (VI)	
CHRONIC EXPOSURE								
Systemic								
26	Human	7 yr avg 5 d/wk 8 hr/d (occup)	Renal		0.05 M (increase in retinol binding protein and tubular antigen)		Franchini and Mutti 1988 CrO3 (VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
27	Human	>1 yr (occup)	Resp		0.025 M (bleeding nasal septum)		Gibb et al. 2000a CrO3 (VI)	
			Ocular		0.049 M			
28	Human	(occup)	Resp		0.414 (nasal septum perforation, chronic pharyngitis, atrophy of larynx)		Hanslian et al. 1967 CrO3 (VI)	
			Gastro		0.414 (chronic tonsilitis)			
29	Human	0.2-23.6 yr avg 2.5 yr 5 d/wk 8 hr/d (occup)	Resp		0.002[b] (nasal mucosa atrophy and ulceration, mild decreased lung function)		Lindberg and Hedenstierna 1983 CrO3 (VI)	
30	Human	0.1-26 yr 5.3 yr avg 5 d/wk 8 hr/d (occup)	Renal		0.004 M (increased urinary beta-2-microglobulin)		Lindberg and Vesterberg 1983b CrO3 (VI)	
31	Human	(occup)	Renal		0.0042 (increased prevalence of high N-acetyl-B-glucosamindase levels)		Liu et al. 1998 Cr(VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
32	Human	7.5 yr avg (range 3-16 yr) (occup)	Resp		0.004 M (epitaxis, rhinorrhea, nasal septum ulceration and perforation)		Lucas and Kramkowski 1975 CrO3 (VI)	
			Gastro		0.004 M (stomach pains and cramps, ulcers)			
33	Rat (Wistar)	18 mo 7 d/wk 22 hr/d	Resp	0.1 M			Glaser et al. 1986, 1988 Na2Cr2O7.2H2O (VI)	
			Hemato	0.1 M				
			Hepatic	0.1 M				
			Renal	0.1 M				
			Endocr	0.1 M				
			Bd Wt	0.1 M				
34	Rat (Wistar)	2 yr 4 d/wk 4-5 hr/d	Resp			1.6 (granulomata, giant cells, bronchopneumonia, abscesses)	Steffee and Baetjer 1965 Finely ground chromium roast (VI)	
35	Mouse (C57BL/6)	18 mo 5 d/wk 5.5 hr/d	Resp			4.3 (epithelial necrosis, hyperplasia)	Nettesheim and Szakal 1972 CaCrO4 (VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
36	Gn Pig (NS)	4.5 yr 4 d/wk 4-5 hr/d	Resp			1.6 (alveolar and interstitial inflammation; alveolar hyperplasia, interstitial fibrosis)	Steffee and Baetjer 1965 Mixed chromium roast K2Cr2O7, Na2CrO4 (VI)	
Immuno/ Lymphoret								
37	Human	5.8 yr (Occup)			0.001 (increased response of peripheral blood mononucleocytes to concavalin A)		Mignini et al. 2004 Cr (VI)	
Cancer								
38	Human	1 mo- 29 yr 5 d/wk 8 hr/d (occup)				0.5 M (CEL: lung cancer)	Hayes et al. 1989 PbCrO4 and ZnCrO4 (VI)	
39	Human	4-19 yr 5 d/wk 8 hr/d (occup)				0.5 (CEL: lung cancer)	Langård and Norseth 1975 PbCrO4 and ZnCrO4 (VI)	
40	Human	1-7 yr 5 d/wk 8 hr/d (occup)				0.25 (CEL: lung cancer)	Mancuso 1975 Soluble Cr(VI)	
41	Human	1 mo- 29 yr 5 d/wk 8 hr/d (occup)				0.1 M (CEL: lung cancer)	Sheffet et al. 1982 PbCrO4 and ZnCrO4 (VI)	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
42	Rat (Wistar)	18 mo 7 d/wk 22 hr/d				0.1 M (CEL: lung tumors)	Glaser et al. 1986, 1988 Na2Cr2O7.2H2O (VI)	
43	Mouse (C57BL/6)	18 mo 5 d/wk 5 hr/d				4.3 (CEL: alveologenic adenomas and adenocarcinomas)	Nettesheim et al. 1971 CaCrO4 (VI)	

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

b Used to derive an intermediate and chronic inhalation minimal risk level (MRL) of 0.000005 mg chromium(VI)/m3 for chromium (VI) trioxide and soluble chromium (VI) compounds. Exposure concentration adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for human variability and 10 for extrapolating from a LOAEL).

c Used to derive an intermediate inhalation minimal risk level (MRL) of 0.0003 mg chromium(VI)/m3 for particulate chromium (VI) compounds. Benchmark concentration of 0.016 mg chromium (VI)/m3 was divided by an uncertainty factor of 30 (3 for pharmacodynamic variability between species and 10 for human variability).

(VI) = hexavalent; avg = average; BaCrO4 = barium chromate; BAL = bronchoalveolar lavage; Bd Wt = body weight; CaCrO4 = calcium chromate; Cardio = cardiovascular; CEL = cancer effect level; Cr = chromium; CrCl3 = chromium trichloride; Cr(NO3)3SH2O = chromium nitrate; CrO2 = chromium dioxide; CrO3 = chromium trioxide; Cr2O3 = chromium oxide; Cr2(SO)3 = chromium sulfate; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; K2Cr2O7 = potassium dichromate; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = rnonth(s); Na2CrO4 = sodium chromate; Na2Cr2O7H2O = sodium dichromate dihydrate; (NH4)2Cr2O7 = ammonium dichromate; NS = not specified; NOAEL = no-observed-adverse-effect level; occup = occupational; PbCrO4 = lead chromate; RBC = red blood cell; Resp = respiratory; WBC =white blood cell; wk = week(s); x = times; yr = year(s); ZnCrO4 = zinc chromate

Figure 3-1 Levels of Significant Exposure to Chromium VI - Inhalation

Acute (≤14 days)

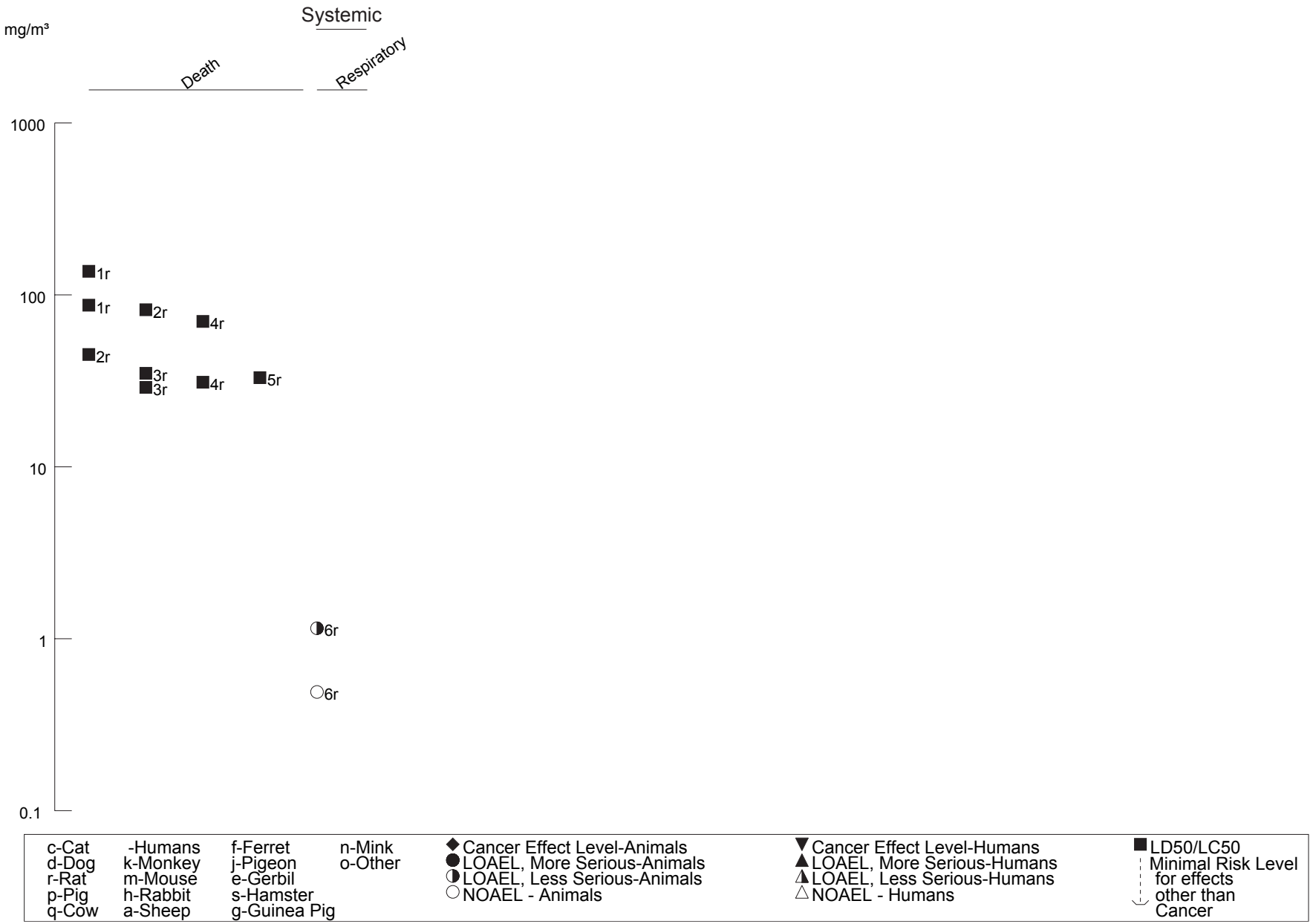


Figure 3-1 Levels of Significant Exposure to Chromium VI - Inhalation *(Continued)*

Intermediate (15-364 days)

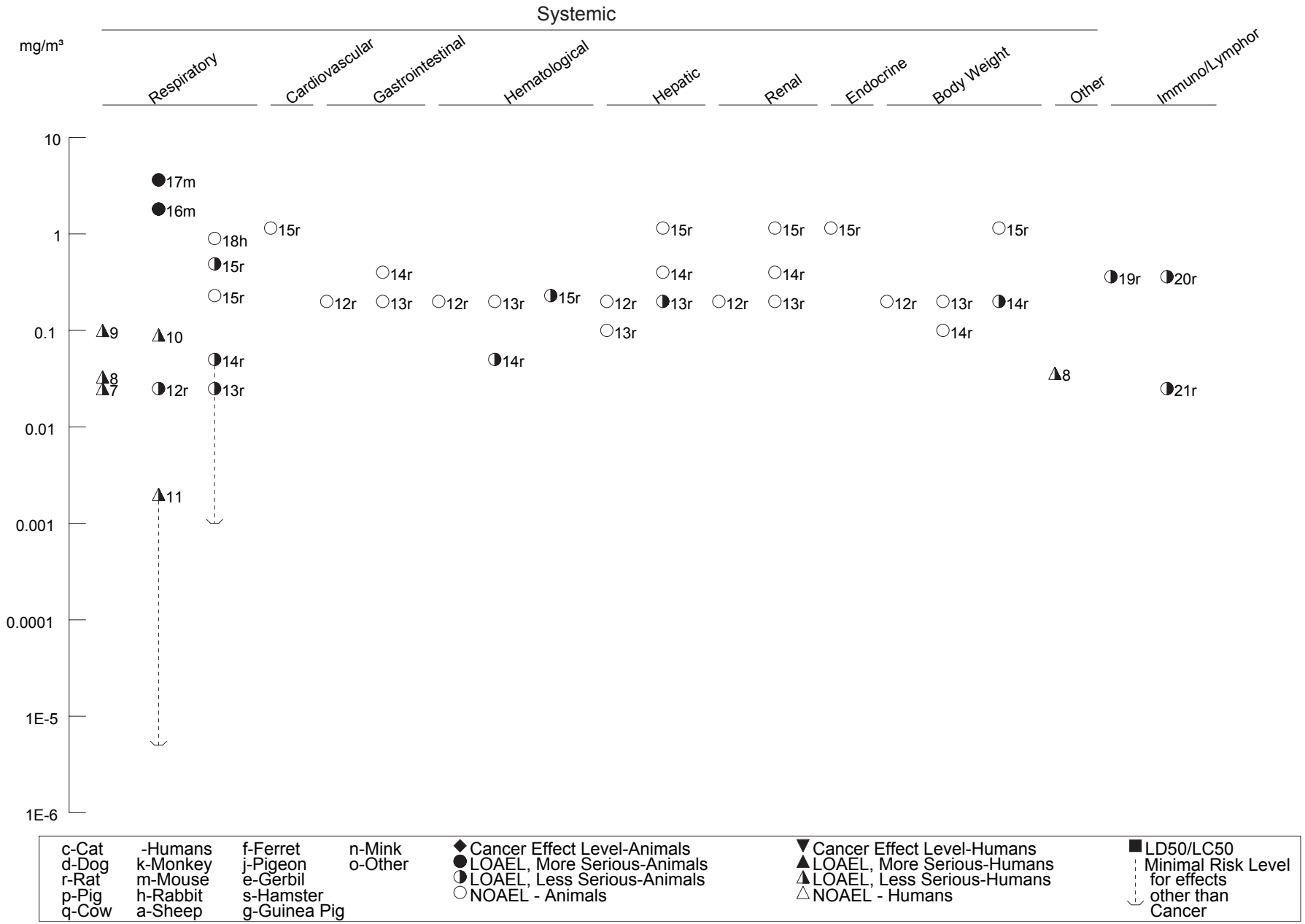


Figure 3-1 Levels of Significant Exposure to Chromium VI - Inhalation *(Continued)*

Intermediate (15-364 days)

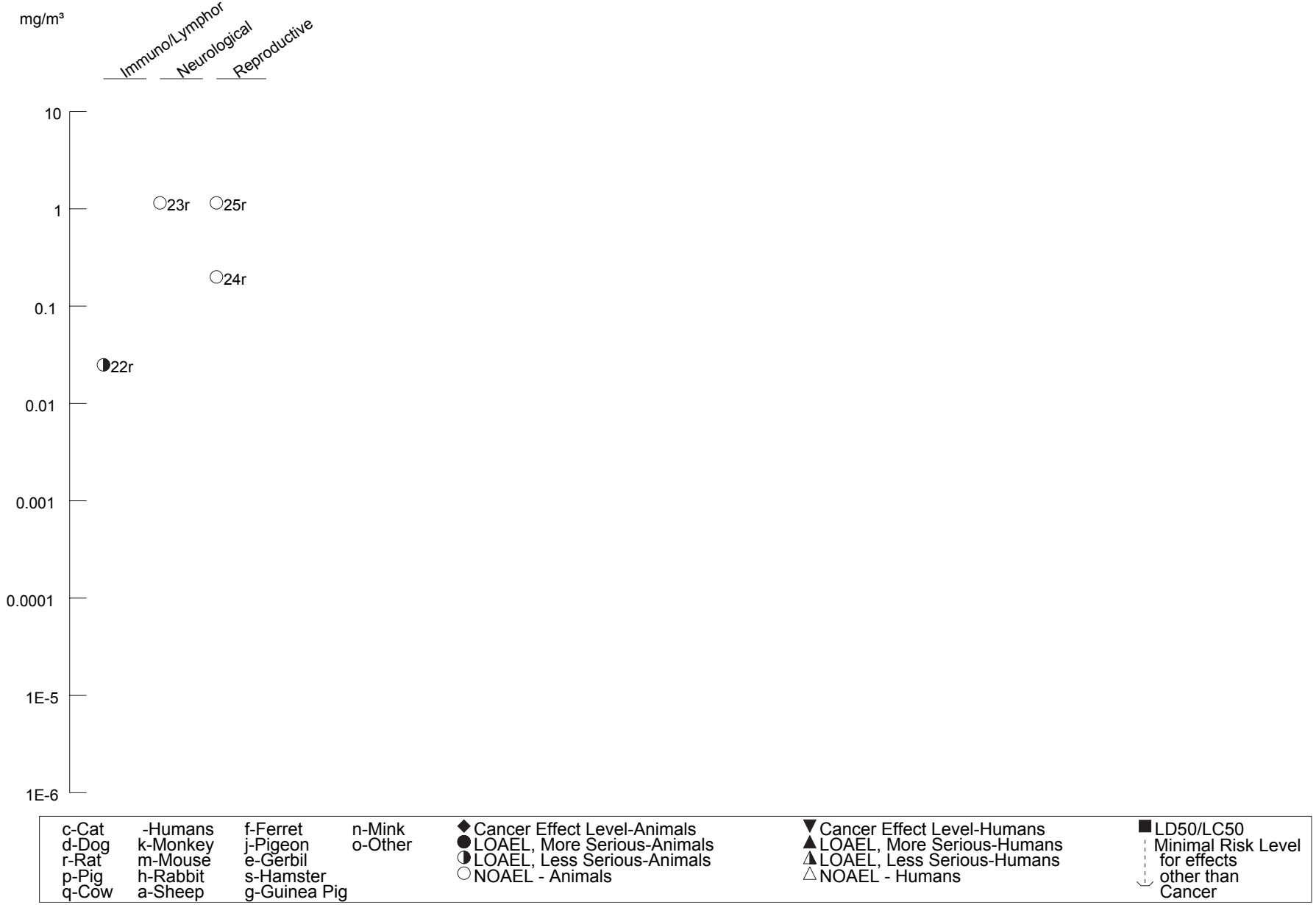
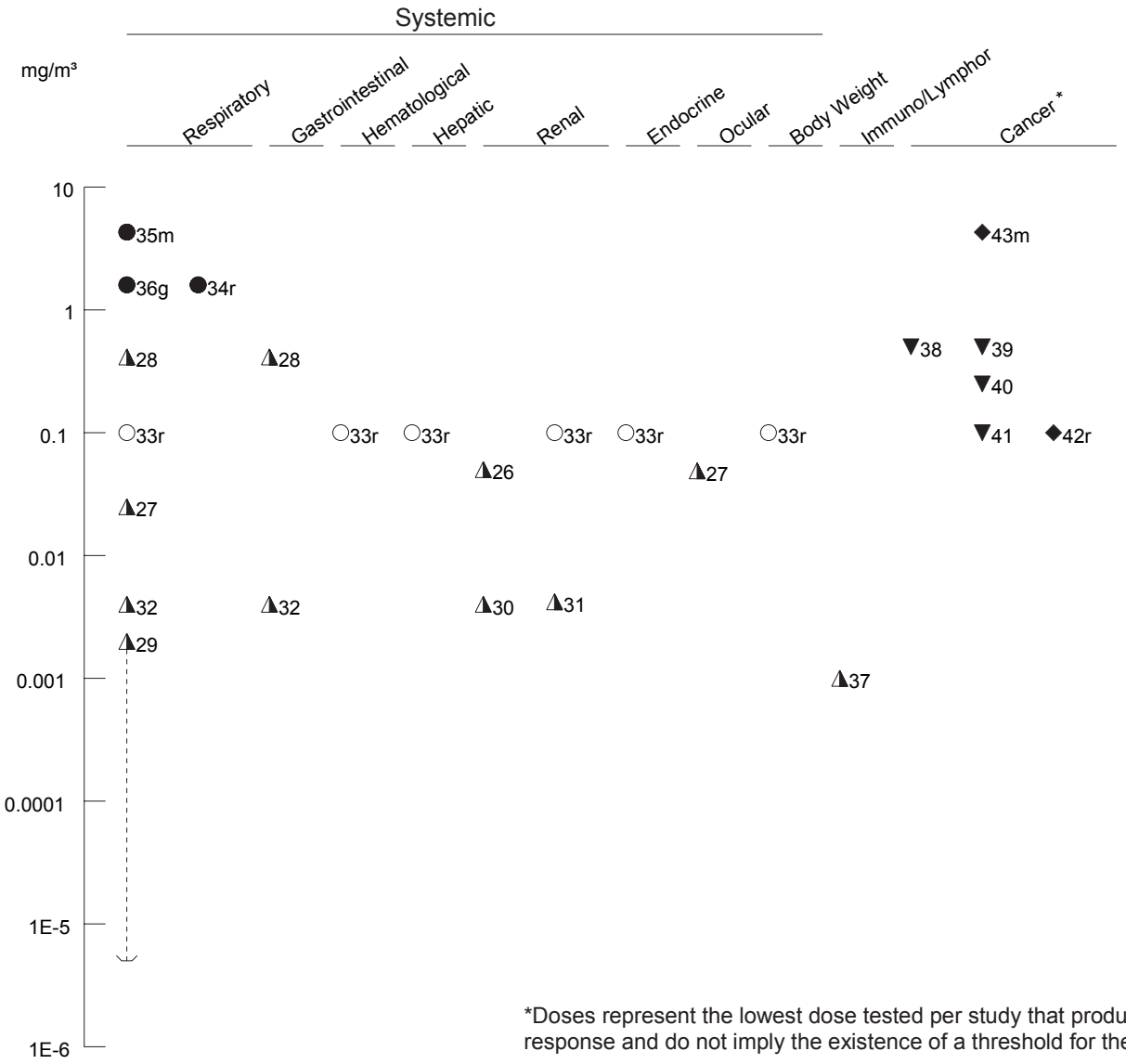


Figure 3-1 Levels of Significant Exposure to Chromium VI - Inhalation *(Continued)*

Chronic (≥365 days)



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

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

Table 3-2 Levels of Significant Exposure to Chromium III - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Hamster (Syrian)	30 min	Resp		0.9 (increased acid phosphatase activity in lung tissue)		Henderson et al. 1979 CrCl3 (III)	
INTERMEDIATE EXPOSURE								
Systemic								
2	Rat (CDF)	13 wk 6 hr/d 5 d/wk	Resp	3 F	3 M[b] (septal cell hyperplasia and interstitial inflammation of the lung; increased absolute and relative lung weight at 30 mg/m3)		Derelanko et al. 1999 Cr2O3 (III)	
					10 F (interstitial inflammation and hyperplasia of alveolar septa)			
			Cardio	30				
			Gastro	30				
			Hemato	30				
			Hepatic	30				
			Renal	30				
			Endocr	30				
			Ocular	30				
			Bd Wt	30				

Table 3-2 Levels of Significant Exposure to Chromium III - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
3	Rat (CDF)	13 wk 6 hr/d 5 d/wk	Resp		3[c] (inflammation of lung; nasal tissues and larynx lesions; increased lung weight)		Derelanko et al. 1999 Cr2(OH)x(SO4)yNaSO4.2H2O (III)	
			Cardio	30				
			Gastro	30				
			Hepatic	30				
			Renal	30				
			Endocr	30				
			Ocular	30				
			Bd Wt	3 M	10 M (~10% decreased in body weight)			
4	Rabbit (NS)	4-6 wk 5 d/wk 6 hr/d	Resp		0.6 M (decreased macrophage activity)		Johansson et al. 1986b Cr(NO3)39H2O(III)	
Immuno/ Lymphoret								
5	Rat (CDF)	13 wk 6 hr/d 5 d/wk			3 (hyperplasia of mediastinal lymph node)		Derelanko et al. 1999 Cr2O3 (III)	
6	Rat (CDF)	13 wk 6 hr/d 5 d/wk			3 (histiocytosis, lymphoid hyperplasia and enlargement of peribronchial and mediastinal lymph nodes)		Derelanko et al. 1999 Cr2(OH)x(SO4)yNaSO4.2H2O (III)	

Table 3-2 Levels of Significant Exposure to Chromium III - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
Neurological								
7	Rat (CDF)	13 wk 6 hr/d 5 d/wk		30			Derelanko et al. 1999 Cr2(OH)x(SO4)yNaSO4.2H2O (III)	
8	Rat (CDF)	13 wk 6 hr/d 5 d/wk		30			Derelanko et al. 1999 Cr2O3 (III)	Increased absolute and relative lung weight in males at 30 mg/m3.
Reproductive								
9	Rat (CDF)	13 wk 6 hr/d 5 d/wk		30			Derelanko et al. 1999 Cr2O3 (III)	
10	Rat (CDF)	13 wk 6 hr/d 5 d/wk		30			Derelanko et al. 1999 Cr2(OH)x(SO4)yNaSO4.2H2O (III)	
CHRONIC EXPOSURE								
Systemic								
11	Human	2-12 yr 5 d/wk 8 hr/d (occup)	Renal	0.075 M			Foa et al. 1988 Cr2O3 (III)	
12	Human	(occup)	Resp	1.99			Korallus et al. 1974a Cr2O3 and Cr2(SO4)3 (III)	

Table 3-2 Levels of Significant Exposure to Chromium III - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
			Hemato	1.99				

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

b Used to derive an intermediate-duration inhalation MRL of 0.005 mg chromium(III)/m3 as insoluble trivalent chromium particulate compounds. The minimal LOAEL of 3 mg chromium(III)/m3 was adjusted for intermittent exposure, converted to a human equivalent concentration (0.43 mg chromium(III)/m3), and divided by a composite uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation from animals to humans and 10 for human variability).

c Used to derive an intermediate-duration inhalation MRL of 0.0001 mg chromium(III)/m3 as soluble trivalent chromium particulate compounds. The LOAEL of 3 mg chromium(III)/m3 was duration-adjusted for intermittent exposure, converted to a human equivalent concentration (0.04 mg chromium(III)/m3) and divided by a composite uncertainty factor of 300 (10 for use of a LOAEL, 3 for variability between animals to humans and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Chromium III - Inhalation

Acute (≤14 days)

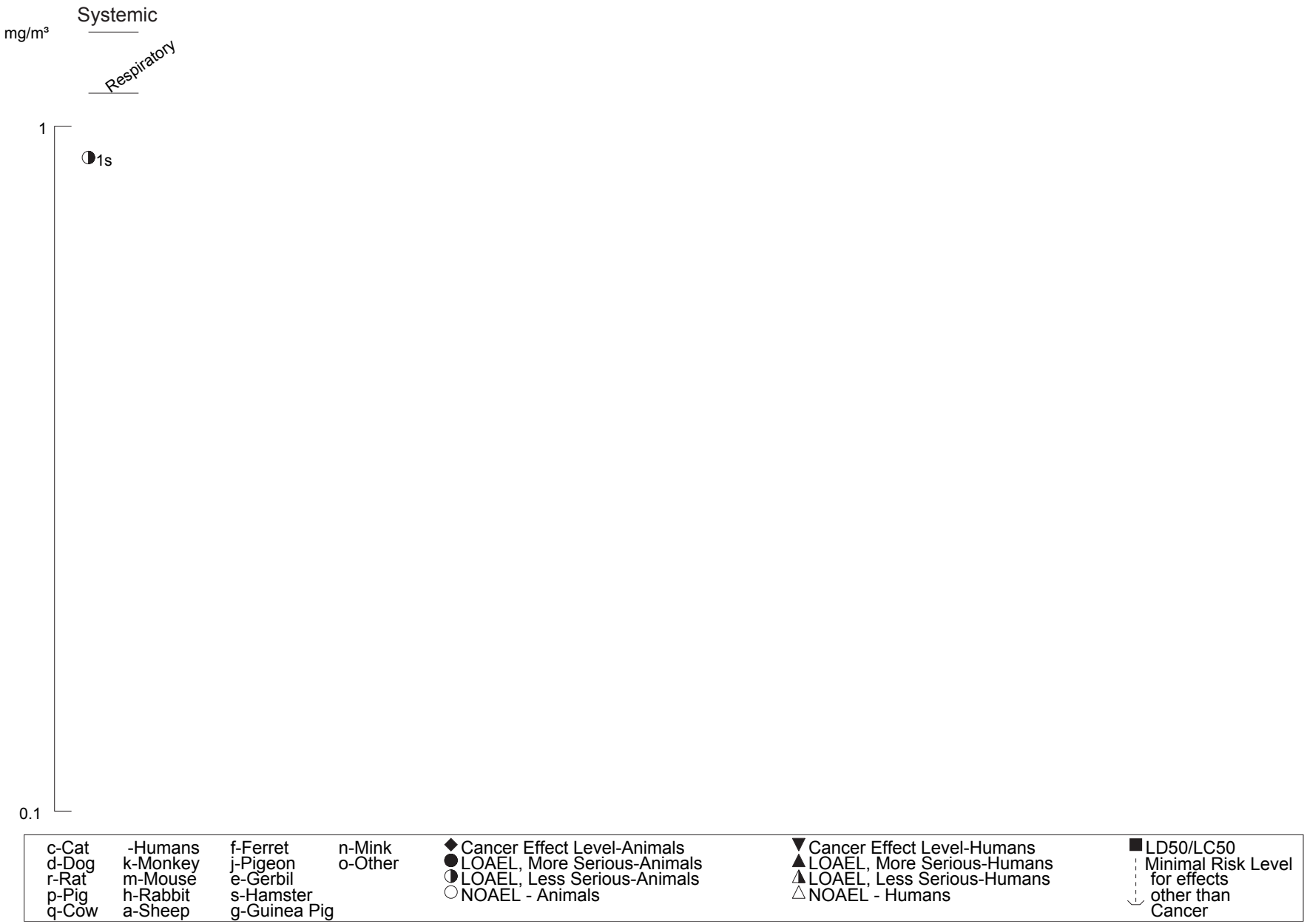


Figure 3-2 Levels of Significant Exposure to Chromium III - Inhalation *(Continued)*

Intermediate (15-364 days)

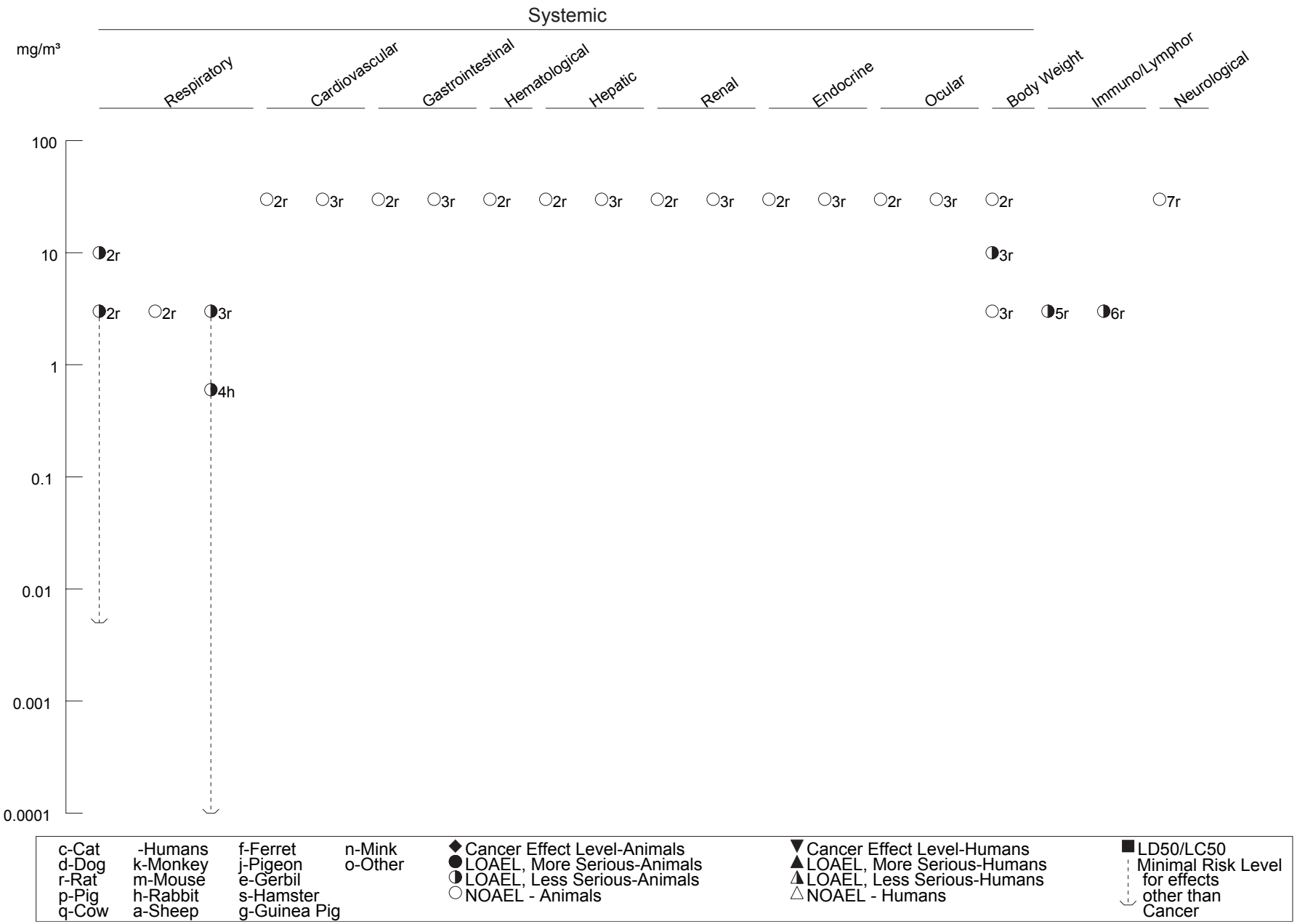


Figure 3-2 Levels of Significant Exposure to Chromium III - Inhalation *(Continued)*

Intermediate (15-364 days)

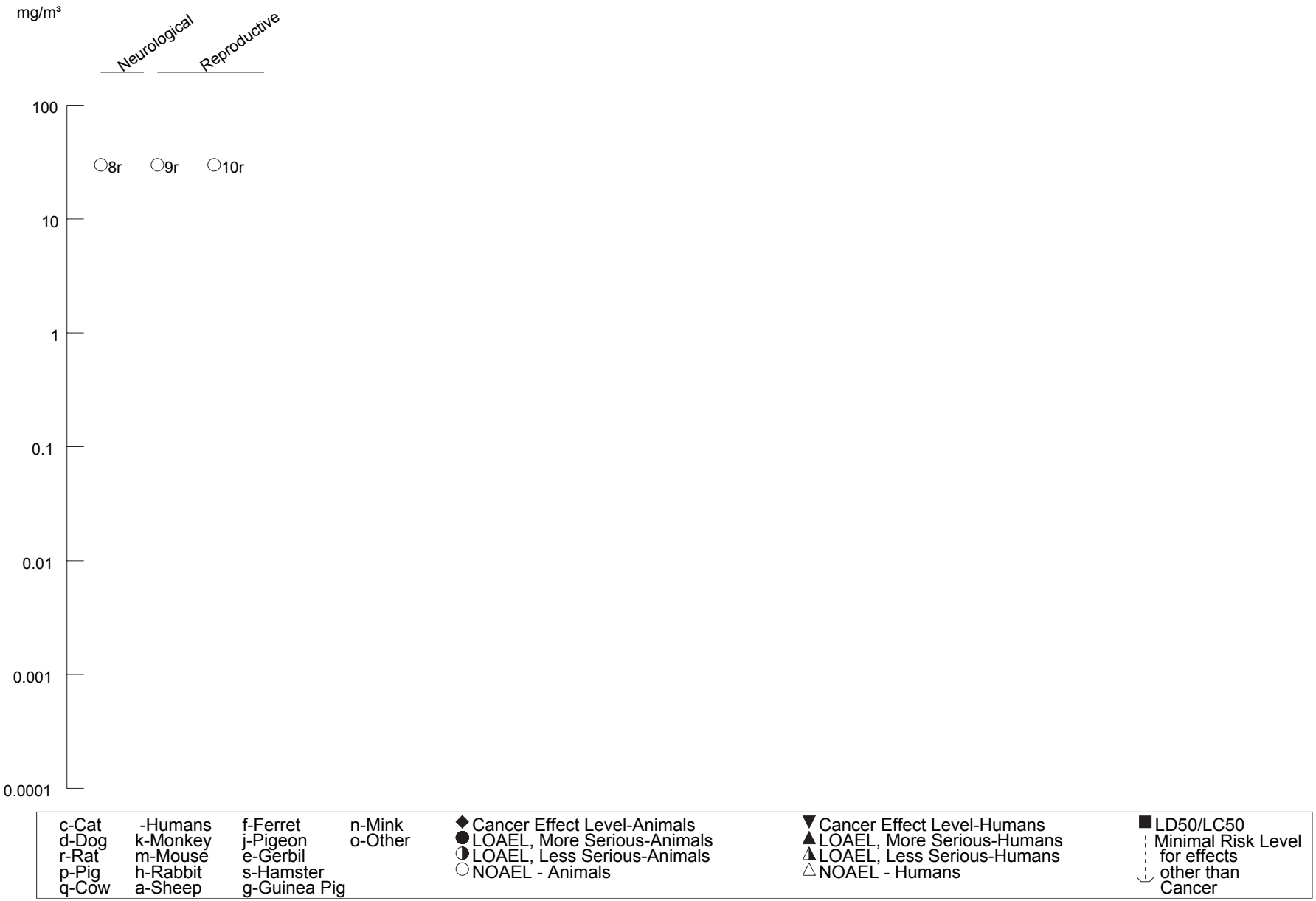
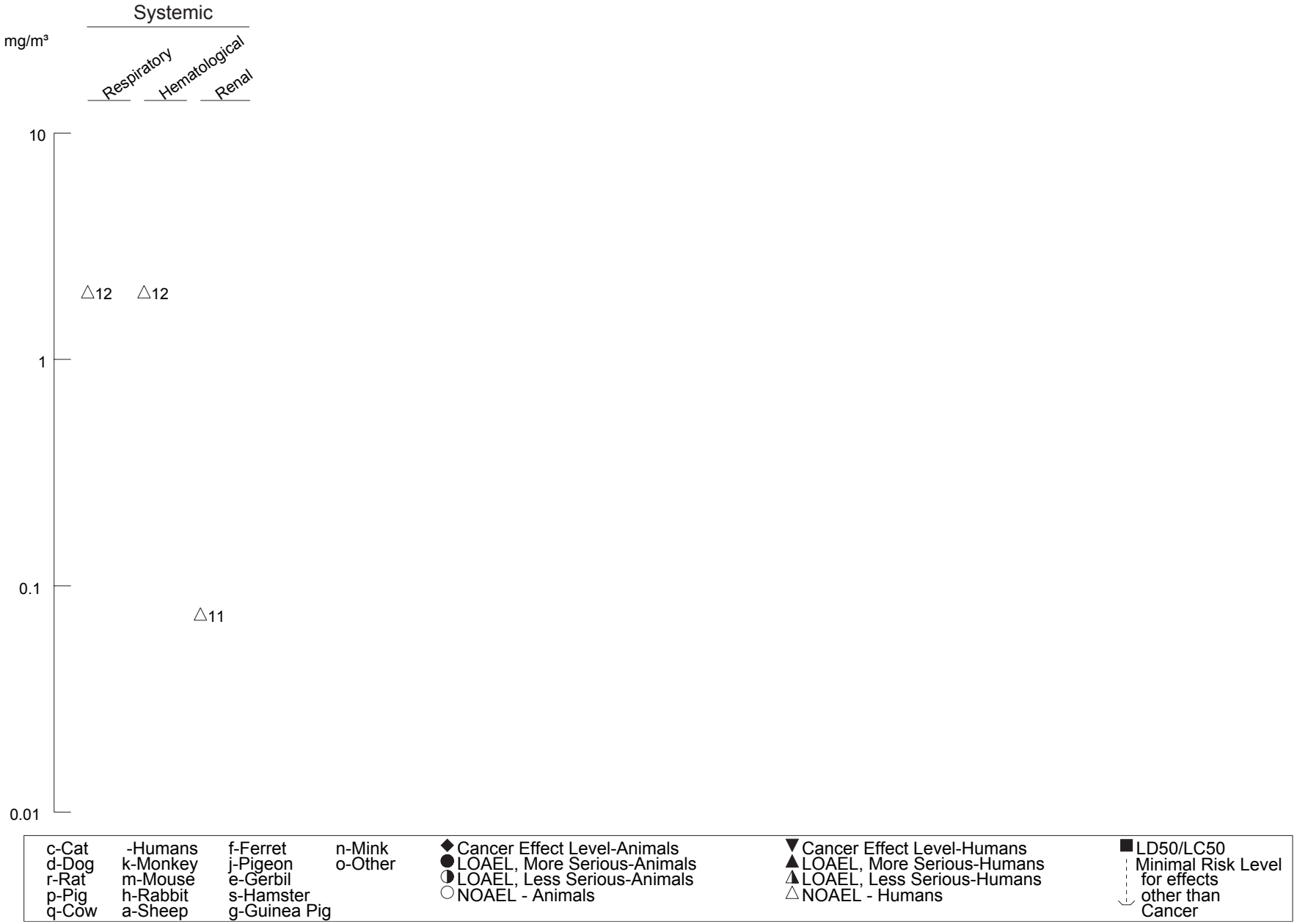


Figure 3-2 Levels of Significant Exposure to Chromium III - Inhalation *(Continued)*

Chronic (≥365 days)



Dyspnea, cough, and wheezing were reported in two cases in which the subjects inhaled "massive amounts" of chromium(VI) trioxide. Marked hyperemia of the nasal mucosa without nasal septum perforation was found in both subjects upon physical examination (Meyers 1950). In a chrome plating plant where poor exhaust resulted in excessively high concentrations of chromium trioxide fumes, workers experienced symptoms of sneezing, rhinorrhea, labored breathing, and a choking sensation when they were working over the chromate tanks. All five of the subjects had thick nasal and postnasal discharge and nasal septum ulceration or perforation after 2–3 months of exposure (Lieberman 1941). Asthma developed in a man who had been well until 1 week after beginning employment as an electroplater. When challenged with an inhalation exposure to a sample of chromium(III) sulfate, he developed coughing, wheezing, and decreased forced expiratory volume. He also had a strong asthmatic reaction to nickel sulfate (Novey et al. 1983). Thus, chromium-induced asthma may occur in some sensitized individuals exposed to elevated concentrations of chromium in air, but the number of sensitized individuals is low and the number of potentially confounding variables in the chromium industry is high.

Intermediate- to chronic-duration occupational exposure to chromium(VI) may cause an increased risk of death due to noncancer respiratory disease. In a retrospective mortality study of 1,288 male and 1,401 female workers employed for at least 6 months in a chrome plating and metal engineering plant in the United Kingdom between 1946 and 1975, a statistically significant excess of death from diseases of the respiratory system (noncancer) were obtained for men (observed/expected [O/E]=72/54.8, standard mortality ratio [SMR]=131, $p<0.05$) and men and women combined (O/E=97/76.4, SMR=127, $p<0.05$), but not for women alone. Exposure was mainly to chromium trioxide, but exposure concentrations were not precisely known. The contribution of nickel exposure to the effects was found to be unimportant, while data on smoking habits were not available (Sorahan et al. 1987). Similarly, a high SMR was found for noncancer respiratory disease among 1,212 male chromate workers who were employed for at least 3 months in three chromate plants in the United States during the years 1937–1960 and followed for 24 years (O/E=19/7.843, SMR=242) (Taylor 1966). The increased risk of death from respiratory effects correlated with duration of employment in chromate production, but no information on exposure levels, smoking habits, or exposure to other chemicals was provided. The nature of the respiratory diseases was not further described in either of these reports. Chromate production workers in the United Kingdom who were first employed before 1945 had a high risk of death from chronic obstructive airways disease (O/E=41/28.66, SMR=143, $p<0.05$) (Davies et al. 1991). Exposure concentrations were not known, and reliable smoking data were not available.

Occupational exposure to chromium(VI) as chromium trioxide in the electroplating industry caused upper respiratory problems. A case history of nine men in a chrome plating facility reported seven cases of nasal septum ulceration. Signs and symptoms included rhinorrhea, nasal itching and soreness, and epistaxis. The men were exposed from 0.5 to 12 months to chromium trioxide at concentrations ranging from 0.09 to 0.73 mg chromium(VI)/m^3 (Kleinfeld and Rosso 1965). Electroplating workers in Sao Paulo, Brazil, exposed to chromium trioxide vapors while working with hot chromium trioxide solutions had frequent incidences of coughing, expectoration, nasal irritation, sneezing, rhinorrhea, and nose-bleed and developed nasal septum ulceration and perforation. The workers had been employed for <1 year, and most of the workers had been exposed to concentrations >0.1 mg chromium(VI)/m^3 (Gomes 1972). Nose and throat irritation, rhinorrhea, and nose-bleed also occurred at higher incidence in chrome platers in Singapore than in controls (Lee and Goh 1988).

Numerous studies of workers chronically exposed to chromium(VI) compounds have reported nasal septum perforation and other respiratory effects. Workers at an electroplating facility exposed to 0.0001–0.0071 mg chromium(VI)/m^3 as chromium trioxide for an average of 26.9 months complained of excessive sneezing, rhinorrhea, and epistaxis. Many of the workers had ulcerations and/or perforations of the nasal mucosa (Cohen et al. 1974). A study using only questionnaires, which were completed by 997 chrome platers and 1,117 controls, found a statistically significant increase in the incidence of chronic rhinitis, rhinitis with bronchitis, and nasal ulcers and perforations in workers exposed to chromium(VI) in the chrome plating industry in 54 plants compared to the control population (Royle 1975b). The workers had been exposed to chromium(VI) in air and in dust. The air levels were generally <0.03 mg chromium(VI)/m^3, and dust levels were generally between 0.3 and 97 mg chromium(VI)/g. The exposure levels at which effects first occurred could not be determined. A NIOSH Health Hazard Evaluation of an electroplating facility in the United States reported nasal septum perforation in 4 of 11 workers employed for an average of 7.5 years and exposed to mean concentrations of 0.004 mg chromium(VI)/m^3. Many of the workers had epistaxis, rhinitis, and nasal ulceration (Lucas and Kramkowski 1975). Nasal mucosal changes ranging from irritation to perforation of the septum were found among 77 employees of eight chromium electroplating facilities in Czechoslovakia where the mean level in the breathing zone above the plating baths was 0.414 mg chromium(VI)/m^3 (Hanslian et al. 1967). The incidence of olfactory cleft obstruction, dry nose, feelings of nasal obstruction, and nasal crusting was significantly increased in workers employed at chromium plating factories (mean employment duration of 7.9 years) in An-San, Korea compared to an unexposed control group (Kitamura et al. 2003). Air concentrations of chromium(VI) ranged from 0.005 to 0.03 mg chromium(VI)/m^3 and of chromium(III) ranged from 0.005 to 0.06 mg chromium(III)/m^3. Increased incidences of nasal septum

perforation, nasal septum ulcer, and nasal obstruction were observed in workers at chromium electroplating facilities exposed for a mean duration of 6.1 years, as compared to workers at zinc electroplating facilities (Kuo et al. 1997a). The chromium electroplating workers had 31.7 and 43.9 times greater risks of developing nasal septum ulcers or nasal perforations, respectively, than the zinc workers. A significant relationship between duration of exposure and the risk of nasal septum ulcers was also found; the chromium electroplating workers with a work duration of >9 years had a risk 30.8 times higher than those with a work duration of <2 years. Duration did not significantly affect the risk of nasal perforation. Statistically significant decreases in vital capacity, forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV_1) were also observed in the chromium workers. Alterations in lung function were also reported in a study of 44 workers at 17 chromium electroplating facilities (Bovet et al. 1977). Statistically significant decreases in forced expiratory volume in 1 second and forced expiratory flow were observed; vital capacity was not altered. Lower lung function values were found among workers with high urinary chromium levels (exposure levels were not reported), and it was determined that cigarette smoking was not a confounding variable.

A study of respiratory effects, lung function, and changes in the nasal mucosa in 43 chrome plating workers in Sweden exposed to chromium(VI) as chromium trioxide for 0.2–23.6 years (median=2.5 years) reported respiratory effects at occupational exposure levels of 0.002 mg chromium(VI)/m^3. Signs and symptoms of adverse nasal effects were observed and reported at mean exposure levels of 0.002–0.2 mg chromium(VI)/m^3. Effects noted at ≤0.002 mg chromium(VI)/m^3 included a smeary and crusty septal mucosa and atrophied mucosa. Nasal mucosal ulceration and septal perforation occurred in individuals exposed at peak levels of 0.02–0.046 mg chromium(VI)/m^3; nasal mucosal atrophy and irritation occurred in individuals exposed at peak levels of 0.0025–0.011 mg chromium(VI)/m^3; and no significant nasal effects were observed in individuals exposed at peak levels of 0.0002–0.001 mg chromium(VI)/m^3. Workers exposed to mean concentrations of 0.002–0.02 mg chromium(VI)/m^3 had slight, transient decreases in FVC, forced expired volume in 1 second (FEV_1), and forced mid-expiratory flow during the workday. Workers exposed to <0.002 mg chromium(VI)/m^3 showed no effects on lung function (Lindberg and Hedenstierna 1983). The concentrations at which minor lung function changes were observed (0.002–0.02 mg chromium(VI)/m^3) and those at which no changes were observed (<0.002 mg chromium(VI)/m^3) are similar to those for nasal effects (0.0025–0.011 mg chromium(VI)/m^3). The effects observed in this study may not have resulted from exposure levels actually measured, but may have resulted from earlier exposure under unknown conditions. Furthermore, poor personal hygiene practices resulting in transfer of chromium(VI) in chrome plating solutions from the hands to the nose could contribute to the development of nasal ulceration and

perforation (Cohen et al. 1974; Lucas and Kramkowski 1975), perhaps leading to an underestimation of airborne levels of chromium(VI) necessary to cause these effects. Despite these considerations, the study by Lindberg and Hedenstierna (1983) is useful because it indicates concentration-responses of chromium(VI) compounds that cause significant nasal and respiratory effects. The LOAEL of 0.002 mg chromium(VI)/m^3 for respiratory effects in humans was used to calculate an inhalation MRL of $5x10^{-6}$ mg chromium(VI)/m^3 for intermediate-duration exposure to chromium(VI) as chromium trioxide mists and other dissolved hexavalent chromium aerosols or mists as described in the footnotes in Table 3-1.

Occupational exposure to chromium(VI) and/or chromium(III) in other chromium-related industries has also been associated with respiratory effects. These industries include chromate and dichromate production, stainless steel welding, and possibly ferrochromium production and chromite mining.

In a survey of a facility engaged in chromate production in Italy, where exposure concentrations were ≥0.01 mg chromium(VI)/m^3, high incidences of nasal septum perforation, septal atrophy and ulcerations, sinusitis, pharyngitis, and bronchitis were found among 65 men who worked in the production of dichromate and chromium trioxide for at least 1 year (Sassi 1956). Medical records of 2,307 male workers (all nonsmokers) employed at a chromate production plant in Baltimore, Maryland between 1950 and 1974 were evaluated to determine the percentage of workers reporting clinical symptoms, mean time of employment to first diagnosis of symptoms, and mean exposure to chromium(VI) at the time of first diagnosis (exposure for each worker was the annual mean in the area of employment during the year of first diagnosis) (Gibb et al. 2000a). The most frequently reported clinical symptoms were irritation and ulcerated nasal septum, occurring in 68.1 and 62.9% of the cohort, respectively. For irritation of the nasal septum, the mean time of employment to first diagnosis was 89 days and the mean annual exposure level during the year of first diagnosis was 0.025 mg chromium(VI)/m^3; for nasal septal ulceration, the mean time of employment to first diagnosis was 86 days and the mean annual exposure level during the year of first diagnosis was 0.028 mg chromium(VI)/m^3. Other nasal effects had a longer time to first diagnosis. The time to first diagnosis for perforated nasal septum was 313 days, occurring in 17.3% of the cohort at a mean exposure level of 0.033 mg chromium(VI)/m^3, and for bleeding nasal septum, the time to first diagnosis was 418 days, occurring in 12.1% of the cohort at a mean exposure level of 0.025 mg chromium(VI)/m^3. In a study of 97 workers from a chromate plant exposed to a mixture of insoluble chromite ore containing chromium(III) and soluble chromium(VI) as sodium chromate and dichromate, evaluation for respiratory effects revealed that 63% had perforations of the nasal septum, 86.6% had chemical rhinitis, 42.3% had chronic chemical pharyngitis, 10.35% had laryngitis, and 12.1% had sinus, nasal, or laryngeal polyps. The number of complaints and clinical signs increased as the exposure to

respirable chromium(VI) and chromium(III) compounds increased, but exposure levels at which effects first occurred were not clearly defined (Mancuso 1951). An extensive survey to determine the health status of chromate workers in seven U.S. chromate production plants found that effects on the lungs consisted of bilateral hilar enlargement. Various manufacturing processes in the plants resulted in exposure of workers to chromite ore (mean time-weighted concentration of 0–0.89 mg chromium(III)/m^3); water-soluble hexavalent chromium compounds (0.005–0.17 mg chromium(VI)/m^3); and acid-soluble/water-insoluble chromium compounds (including basic chromium sulfate), which may or may not entirely represent trivalent chromium (0–0.47 mg chromium/m^3) (PHS 1953). Challenge tests with fumes from various stainless steel welding processes indicated that the asthma observed in two stainless steel welders was probably caused by chromium or nickel, rather than by irritant gases (Keskinen et al. 1980). In a study of 54 male miners in Zimbabwe exposed to chrome ore dust, decreases in pulmonary function, as indicated by measures of FVC, FEV_1, peak expiratory flow rate (PEFR), and FEV_1%, was observed compared to an unexposed control (e.g., non-mining) population (Osim et al. 1999). Exposure levels were reported only as respirable dust, not as chromium specifically, and the mining company did not employ industrial hygiene practices to reduce exposure. In this same study, no changes in lung function were observed in a group of 46 male miners working for a company following industrial hygiene procedures (again, specific chromium exposure levels were not reported). The analysis controlled for smoking and infectious respiratory diseases. In a report of 10 cases of pneumoconiosis in underground workers in chromite mines in South Africa, radiographic analysis revealed fine nodulation and hilar shadows. Chromium in the chromite ore in South Africa was in the form of chromium(III) oxide. The cause of the pneumoconiosis was considered to be deposition of insoluble radio-opaque chromite dust in the tissues, rather than fibrosis (Sluis-Cremer and du Toit 1968). In a case report of a death of a sandblaster in a ferrochromium department of an iron works, the cause of death was silicosis, but autopsy also revealed diffuse enlargement of alveolar septae and chemical interstitial and alveolar chronic pneumonia, which were attributed to inhalation of chromium(III) oxide (Letterer 1939). In an industrial hygiene survey of 60 ferrochromium workers exposed to chromium(III) and chromium(VI) (0.02–0.19 mg total chromium/m^3) conducted in 1975, appreciably higher incidences of subjective symptoms of coughing, wheezing, and dyspnea were reported compared with controls. These workers had been employed at the plant for at least 15 years. The control group consisted of workers employed at the same plant for <5 years. Statistically significant decreased mean FVC ($p<0.01$) and FEV_1 ($p<0.05$) were found in the ferrochromium workers compared with controls. Two of the ferrochromium workers had nasal septum perforations, which were attributed to previous exposure to hexavalent chromium. A major limitation of this study is that the control group was significantly younger than the study cohort. In addition, the weekly amount of tobacco smoked by the control group was slightly greater than that

smoked by the study groups, and the controls began smoking 5 years earlier than the study groups. Therefore, the increase in subjective respiratory symptoms and decreased pulmonary function parameters cannot unequivocally be attributed to chromium exposure (Langård 1980). However, no increase in the prevalence of respiratory illness was found in a study of 128 workers from two factories that produced chromium(III) oxide or chromium(III) sulfate (Korallus et al. 1974b) or in 106 workers at a factory that produced these chromium(III) compounds where workroom levels were ≤1.99 mg chromium(III)/m^3 (Korallus et al. 1974a). Similar results were reported in a cross-sectional study that was conducted to determine whether occupational exposure to trivalent chromium or hexavalent chromium caused respiratory diseases, decreases in pulmonary function, or signs of pneumoconiosis in stainless steel production workers (Huvinen et al. 1996). The median personal exposure levels were 0.0005 μg/m^3 for chromium(VI) and 0.022 μg/m^3 for chromium(III); the 221 workers were employed for >8 years with an average potential exposure of 18 years. Spirometry measurements were taken and chest radiographic examinations were conducted. There were no significant differences in the odds ratios between the exposed workers and the 95 workers in the control group. The deficits in lung function shown in both populations could be explained by age and smoking habits. In a follow-up study of these workers (Huvinen et al. 2002a), no adverse respiratory effects were observed (as assessed by spirometry, chest x-ray ,and self-reported symptoms) in workers in the chromium(VI) group (n=104) compared to controls (n=81). Workers exposed to chromium(III) in the sintering and crushing departments (n=68) reported an increase in respiratory symptoms (phlegm production, shortness of breath on exertion) compared to control, but no differences in spirometry or chest x-ray. Workers exposed to chromium(III) as chromite ore (n=31) had lower lung function tests, although smoking was a confounding factor. In addition to chromium, workers were also exposed to nickel and molybdenum. In a study of stainless steel workers (all nonsmokers) exposed for a minimum of 14 years to chromium(VI) (n=29), chromium(III) (n=14), or chromite(III) ore (n=5), no increase was observed in the incidence of nasal diseases or nasal symptoms in exposed chromium-exposed workers compared to a control population of 39 workers (Huvinen et al. 2002b). However, although an exposure-related increase in the incidence of clinical signs of nasal irritation was not observed, anterior rhinoscopy revealed a slight increase in the incidence of inflammatory changes in the nasal mucosa of workers exposed to chromium(VI) (risk ratio=2.4) or chromium(III) (risk ratio=2.3), compared to control. The mean exposure level for the chromium(VI) group was 0.5 μg Cr(VI)/m^3, for the chromium(III) group was 248 μg total Cr/m^3 (concentration of chromium(III) not reported) and for the chromite ore group was 22 μg Cr(III)/m^3.

The respiratory system in animals is also a primary target for acute- and intermediate-duration inhalation exposure to chromium(VI) and chromium(III). Rats exposed to sodium dichromate for 28 or 90 days had

increased lung weight but no histopathological abnormalities at concentrations ≤0.2mg chromium(VI)/m^3. The percentage of lymphocytes was increased in the bronchoalveolar lavage fluid at ≥0.025 mg/m^3. A decrease in macrophage activity was observed in the 0.2 mg chromium(VI)/m^3 group exposed for 90 days. Clearance of iron oxide from the lungs decreased in rats exposed to 0.2 mg chromium(VI)/m^3 for 42 days prior to and 49 days after challenge with iron oxide particles when compared to controls. The decreased clearance of iron oxide correlated with the decrease in macrophage activity (Glaser et al. 1985). In a similar but more extensive study, obstructive respiratory dyspnea was observed in rats exposed to sodium dichromate at ≥0.2 mg chromium(VI)/m^3 for 30 or 90 days, and mean lung weight was increased at ≥0.05 mg chromium(VI)/m^3. Slight hyperplasia was observed at high incidence in rats at ≥0.05 mg chromium(VI)/m^3. Lung fibrosis occurred at low incidence in the rats exposed to ≥0.1 mg chromium(VI)/m^3 for 30 days, but not in the 0.05 mg/m^3 or the control groups. The incidence of both these lesions declined after longer exposure, indicating repair. Accumulation of macrophages and inflammation occurred at ≥0.05 mg chromium(VI)/m^3 regardless of duration. Results of bronchoalveolar lavage (BAL) analysis provided further evidence of an irritation effect that was reversible (Glaser et al. 1990). The data from the Glaser et al. (1990) study was used to develop benchmark concentrations (BMCs) (Malsch et al. 1994). The BMC of 0.016 mg chromium(VI)/m^3 for alterations in lactate dehydrogenase levels in BAL fluid was used to calculate an inhalation MRL of 0.0003 mg chromium(VI)/m^3 for intermediate-duration exposure to chromium(VI) as particulate hexavalent compounds as described in the footnote of Table 3-1.

Male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide mist developed nasal hemorrhage after 10 days (lasting for 4 weeks) during a 90-day inhalation study (Kim et al. 2004). "Peculiar sounds" during respiration were noted starting after 1 week of exposure and resolving by week 8 in rats exposed to ≥0.23 mg chromium(VI)/m^3; however, no additional information on this observation was reported. After 90 days, histopathological changes to respiratory tissue included macrophage aggregation and foamy cells, and inflammation of alveolar regions; however, no abnormalities were observed in nasal tissue at 0.49 mg chromium(VI)/m^3 (incidence data were not reported). Mice exposed to chromium trioxide mist at concentrations of 1.81 and 3.63 mg chromium(VI)/m^3 intermittently for ≤12 months developed perforations in the nasal septum, hyperplastic and metaplastic changes in the larynx, trachea, and bronchus, and emphysema (Adachi 1987; Adachi et al. 1986).

The respiratory effects of chromium(III) compounds were investigated in male and female CDF rats exposed to insoluble chromic oxide or soluble basic chromium sulfate by nose-only inhalation at 3, 10, or

30 mg chromium(III)/m^3 for 6 hours/day, 5 days/week for 13 weeks (Derelanko et al. 1999). After 5 days of exposure, BAL was conducted on a subgroup of animals. In rats treated with chromic oxide, a yellow crystalline material was observed in the cytoplasm of mononuclear cells of all exposure groups; however, it is not clear if this observation represents an adverse effect. No other BAL parameters were affected (nucleated cell count and differential, protein and BAL fluid activities of β-glucuronidase, lactic dehydrogenase, and glutathione reductase). In rats treated with basic chromium sulfate, BAL fluid analysis showed significant decreases in nucleated cells at all doses in males and females and decreases in the percentage of segmented neutrophils and mononuclear cells at 30 mg chromium(III)/m^3 in males. Increased amounts of cell debris and lysed cells were present in all basic chromium sulfate groups (incidence data were not reported). In rats exposed to chromic oxide for 13 weeks, absolute and relative lung weights were increased by 12 and 13%, respectively, in males exposed to 30 mg chromium(III)/m^3 as chromic oxide; no change was observed in females. Histopatholgical examination of respiratory tissues showed pigmented macrophages containing a dense black substance, presumably the test substance, throughout the terminal bronchioles and alveolar spaces in rats from all treatment groups; this finding is consistent with normal physiological clearance mechanisms for particulates deposited in the lung and is not considered to be adverse. At concentrations of 10 and 30 mg chromium(III)/m^3, trace to mild chronic interstitial inflammation, characterized by inflammatory cell infiltrates, and septal cell hyperplasia was observed. No lesions were observed in the nasal cavity. Following a 13-week recovery period, microscopic examination of respiratory tissues of rats treated with chromic oxide showed pigmented macrophages and black pigment in peribronchial tissues and the mediastinal lymph node in all treatment groups and septal cell hyperplasia and chronic interstitial inflammation of the lung, both trace-to-mild in severity, in males of all treatment groups and in females exposed to 10 and 30 mg chromium(III)/m^3. In rats treated with basic chromium sulfate, a dose-related increase in absolute and relative lungs weights was observed in all treatment groups. Histopathological examination of respiratory tract tissues revealed chronic inflammation of the lung (characterized by cell infiltration and debris in alveolar spaces and intense inflammation) and alveolar wall hyperplasia in all treatment groups. In addition, inflammation and suppurative and mucoid exudates of nasal tissues and granulomatous inflammation of the larynx were observed in all treatment groups. Incidence data for histopathological findings were not reported. Following the 13-week recovery period for rats treated with basic chromium sulfate, enlargement of the mediastinal lymph node was observed on gross necropsy in all treatment groups. Microscopic examination of respiratory tissues showed changes to the lung (chronic alveolar inflammation, interstitial inflammation, septal cell hyperplasia, and granulomatous inflammation) in all treatment groups, larynx (granulomatous inflammation) in the 10 and 30 mg chromium(III)/m^3 groups, nasal tissues (trace suppurative exudates) in one to two animals in each groups, and mediastinal lymph

node (histiocytosis and hyperplasia) in all treatment groups chromium(III)/m^3 groups. Results of this study demonstrate differences in the respiratory effects of inhaled chromium oxide and inhaled basic chromium sulfate. Effects of soluble basic chromium sulfate were more severe and were observed throughout the respiratory tract, while effects of chromic oxide were more mild and limited to the lung; these observations may be related to differences in chemical-physical properties of the test compounds. Data from the Derelanko et al. (1999) study was used as the basis for intermediate-duration inhalation MRLs for chromium(III) compounds. Since soluble and insoluble chromium(III) compounds exhibited different effects in the respiratory tract, distinct intermediate-duration MRLs were derived for insoluble and soluble trivalent chromium particulates. For insoluble chromium(III) compounds (chromic oxide), the minimal LOAEL of 3 mg chromium(III)/m^3 was used to calculate an intermediate-duration inhalation MRL of 0.005 mg chromium(III)/m^3 for exposure to trivalent chromium particulates as described in the footnote of Table 3-2. For soluble chromium(III) (basic chromium sulfate) compounds, the LOAEL of 3 mg chromium(III)/m^3 was used to calculate an intermediate-duration inhalation MRL of 0.0001 mg chromium(III)/m^3 for exposure to trivalent chromium particulates as described in the footnote of Table 3-2.

Pulmonary fluid from hamsters exposed to 0.9 or 25 mg chromium(III)/m^3 as chromium trichloride for 30 minutes revealed sporadic changes in activities of acid phosphatase and alkaline phosphatase in the lavage fluid at 25 mg chromium(III)/m^3. In the lung tissue, a 75% increase in the acid phosphatase activity was found at 0.9 mg chromium(III)/m^3 and in the β-glucuronidase activity at an unspecified concentration. Histological examination revealed alterations representing mild nonspecific irritation but no morphological damage (Henderson et al. 1979). In rabbits exposed to 0.6 mg chromium(III)/m^3 as chromium nitrate intermittently for 4–6 weeks, changes in the lungs were confined to nodular accumulations of macrophages in the lungs. Macrophage morphology demonstrated black inclusions and large lysosomes. These changes represent normal physiological responses of the macrophages to the chromium particle. Phagocytosis and the reduction of nitroblue tetrazolium to formazan was impaired by chromium(III), indicating a decrease in the functional and metabolic activity of the macrophage (Johansson et al. 1986a, 1986b).

Chronic exposure to chromium(VI) compounds and mixtures of chromium(VI) and chromium(III) compounds have also resulted in adverse respiratory effects in animals. Experiments in which rats were exposed to either chromium(VI) alone as sodium dichromate or a 3:2 mixture of chromium(VI) trioxide and chromium(III) oxide for 18 months showed similar loading of macrophages and increases in lung weight. However, histopathology of rats exposed to 0.1 mg/m^3 of chromium(III) and chromium(VI)

together revealed interstitial fibrosis and thickening of the septa of the alveolar lumens due to the large accumulation of chromium in the lungs, whereas histopathology of the lungs was normal in rats exposed only to chromium(VI) (Glaser et al. 1986, 1988). Mice exposed to 4.3 mg chromium(VI)/m^3 as calcium chromate dust intermittently for 18 months had epithelialization of alveoli. Histopathology revealed epithelial necrosis and marked hyperplasia of the large and medium bronchi, with numerous openings in the bronchiolar walls (Nettesheim and Szakal 1972). Significantly increased incidences of pulmonary lesions (lung abscesses, bronchopneumonia, giant cells, and granulomata) were found in rats exposed chronically to a finely ground, mixed chromium roast material that resulted in airborne concentrations of 1.6–2.1 mg chromium(VI)/m^3 compared with controls. In the same study, guinea pigs exposed chronically to the chromium roast material along with mists of potassium dichromate or sodium chromate solutions that also resulted in 1.6–2.1 mg chromium(VI)/m^3 had significantly increased incidences of alveolar and interstitial inflammation, alveolar hyperplasia, and interstitial fibrosis, compared with controls. Similarly, rabbits were also exposed and also had pulmonary lesions similar to those seen in the rats and guinea pigs, but the number of rabbits was too small for meaningful statistical analysis (Steffee and Baetjer 1965).

In the only study of chromium(IV) exposure, all rats treated with 0.31 or 15.5 mg chromium(IV)/m^3 as chromium dioxide dust for 2 years had discolored mediastinal lymph nodes and lungs, and dust laden macrophages. Lung weight was increased at 12 and 24 months in the 15.5 mg chromium(IV)/m^3 group (Lee et al. 1989). The increased lung weight and macrophage effects probably represent the increased lung burden of chromium dioxide dust and normal physiological responses of macrophages to dust.

Cardiovascular Effects. Information regarding cardiovascular effects in humans after inhalation exposure to chromium and its compounds is limited. In a survey of a facility engaged in chromate production in Italy, where exposure concentrations were $\geq$0.01 mg chromium(VI)/m^3, electrocardiograms were recorded for 22 of the 65 workers who worked in the production of dichromate and chromium trioxide for at least 1 year. No abnormalities were found (Sassi 1956). An extensive survey to determine the health status of chromate workers in seven U.S. chromate production plants found no association between heart disease or effects on blood pressure and exposure to chromates. Various manufacturing processes in the plants resulted in exposure of workers to chromite ore (mean time-weighted concentration of 0–0.89 mg chromium(III)/m^3); water-soluble chromium(VI) compounds (0.005–0.17 mg chromium(VI)/m^3); and acid-soluble/water-insoluble chromium compounds (including basic chromium sulfate), which may or may not entirely represent trivalent chromium (0–0.47 mg chromium/m^3) (PHS 1953). No excess deaths were observed from cardiovascular diseases and ischemic heart disease in a

cohort of 4,227 stainless steel production workers from 1968 to 1984 when compared to expected deaths based on national rates and matched for age, sex, and calendar time (Moulin et al. 1993). No measurements of exposure were provided. In a cohort of 3,408 individuals who had worked in four facilities that produced chromium compounds from chromite ore in northern New Jersey sometime between 1937 and 1971, where the exposure durations of workers ranged from <1 to >20 years, and no increases in atherosclerotic heart disease were evident (Rosenman and Stanbury 1996). The proportionate mortality ratios for white and black men were 97 (confidence limits 88–107) and 90 (confidence limits 72–111), respectively.

Cardiovascular function was studied in 230 middle-aged workers involved in potassium dichromate production who had clinical manifestations of chromium poisoning (96 with respiratory effects and 134 with gastrointestinal disorders) and in a control group of 70 healthy workers of similar age. Both groups with clinical manifestations had changes in the bioelectric and mechanical activity of the myocardium as determined by electrocardiography, kinetocardiography, rheocardiography, and ballistocardiography. These changes were more pronounced in the workers with respiratory disorders due to chromium exposure than in the workers with chromium-induced gastrointestinal effects. The changes in the myocardium could be secondary to pulmonary effects and/or to a direct effect on the blood vessels and myocardium (Kleiner et al. 1970).

For intermediate-duration exposures, no histopathological changes to the heart were observed in male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide (Kim et al. 2004) or in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months, (Derelanko et al. 1999). No histopathological lesions were found in the hearts of rats exposed chronically to chromium dioxide at 15.5 mg chromium(IV)/m^3 (Lee et al. 1989). Additional information regarding cardiovascular effects in animals after exposure to chromium or chromium compounds was not located.

Gastrointestinal Effects. Gastrointestinal effects have been associated with occupational exposure of humans to chromium compounds. In a report of two cases of acute exposure to "massive amounts" of chromium trioxide fumes, the patients complained of abdominal or substernal pain, but further characterization was not provided (Meyers 1950).

In a NIOSH Health Hazard Evaluation of an electroplating facility in the United States, 5 of 11 workers reported symptoms of stomach pain, 2 of duodenal ulcer, 1 of gastritis, 1 of stomach cramps, and 1 of

frequent indigestion. The workers were employed for an average of 7.5 years and were exposed to mean concentrations of 0.004 mg chromium(VI)/m^3 (Lucas and Kramkowski 1975). These workers were not compared to a control group. An otolaryngological examination of 77 employees of eight chromium electroplating facilities in Czechoslovakia, where the mean level in the breathing zone above the plating baths was 0.414 mg chromium(VI)/m^3, revealed 12 cases of chronic tonsillitis, 5 cases of chronic pharyngitis, and 32 cases of atrophy of the left larynx (Hanslian et al. 1967). In a study of 97 workers from a chromate plant exposed to a mixture of insoluble chromite ore containing chromium(III) and soluble chromium(VI) as sodium chromate and dichromate, gastrointestinal radiography revealed that 10 of the workers had ulcer formation, and of these, 6 had hypertrophic gastritis. Nearly all of the workers breathed through the mouth while at work and swallowed the chromate dust, thereby directly exposing the gastrointestinal mucosa. Only two cases of gastrointestinal ulcer were found in 41 control individuals, who had the same racial, social, and economic characteristics as the chromium-exposed group (Mancuso 1951). In a survey of a facility engaged in chromate production in Italy where exposure concentrations were ≥0.01 mg chromium(VI)/m^3, 15.4% of the 65 workers who worked in the production of dichromate and chromium trioxide for at least 1 year had duodenal ulcers and 9.2% had colitis. The ulcers were considered to be due to exposure to chromium (Sassi 1956). Gastric mucosa irritation leading to duodenal ulcer was found in 21 of 90 workers engaged in the production of chromium salts. Symptoms of gastrointestinal pathology appeared about 3–5 years after the workers' initial contact (Sterekhova et al. 1978). Most of these studies reporting gastrointestinal effects did not compare the workers with appropriate controls. Although the gastrointestinal irritation and ulceration due to exposure to chromium(VI) in air could be due to a direct action of chromium(VI) on the gastrointestinal mucosa from swallowing chromium as a result of mouth breathing (or transfer via hand-to-mouth activity), other factors, such as stress and diet, can also cause gastrointestinal effects. While occupational exposure to chromium(VI) may result in gastrointestinal effects, a lower than expected incidence of death from diseases of the digestive tract was found among a cohort of 2,101 employees who had worked for at least 90 days during the years 1945–1959 in a chromium production plant in Baltimore, Maryland, and were followed until 1977. The rate (O/E=23/36.16, SMR=64) is based on comparison with mortality rates for Baltimore (Hayes et al. 1979). In contrast to findings with chromium(VI) compounds, no indication was found that exposure to chromium(III) resulted in stomach disorders in workers employed in two factories that produced chromium(III) oxide or chromium(III) sulfate (Korallus et al. 1974b).

Information regarding gastrointestinal effects in animals after inhalation exposure to chromium or its compounds is limited. For intermediate-duration exposures, no histopathological changes to gastrointestinal tissues in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic

oxide or basic chromium sulfate for 3 months, (Derelanko et al. 1999). Histological examination of the stomachs of rats exposed to sodium dichromate dihydrate at ≤0.2 mg chromium(VI)/m^3 for 28 or 90 days revealed no abnormalities (Glaser et al. 1985). In mice exposed intermittently to 4.3 mg chromium(VI)/m^3 as calcium chromate for 18 months, small ulcerations in the stomach and intestinal mucosa were reported to occur occasionally, but the incidence in the treated mice or controls and other details regarding these lesions were not reported (Nettesheim et al. 1971). No treatment-related histopathological lesions were found in the stomach, large intestine, duodenum, jejunum, or ileum of rats chronically exposed to chromium dioxide at 15.5 mg chromium(IV)/m^3 (Lee et al. 1989).

Hematological Effects. Hematological evaluations of workers occupationally exposed to chromium compounds have yielded equivocal results. Ninety-seven workers from a chromate plant were exposed to a mixture of insoluble chromite ore containing chromium(III) and soluble sodium chromate and dichromate. Hematological evaluations revealed leukocytosis in 14.4% or leukopenia in 19.6% of the workers. The leukocytosis appeared to be related primarily to monocytosis and eosinophilia, but controls had slight increases in monocytes and occasional increases in eosinophils without leukocytosis. Decreases in hemoglobin concentrations and slight increases in bleeding time were also observed (Mancuso 1951). Whether these hematological findings were significantly different from those seen in controls was not stated, but the effects were attributed to chromium exposure. In a survey of a facility engaged in chromate production in Italy where exposure concentrations were ≥0.01 mg chromium(VI)/m^3, hematological evaluation of workers who worked in the production of dichromate and chromium trioxide for at least 1 year were unremarkable or inconclusive (Sassi 1956). In an extensive survey to determine the health status of chromate workers in seven U.S. chromate production plants, hematological evaluations revealed no effects on red blood cell counts, hemoglobin, hematocrit, or white blood cell counts. The sedimentation rate of red cells was higher than that of controls, but the difference was not statistically significant. Various manufacturing processes in the plants resulted in exposure of workers to chromite ore (mean time-weighted concentration of 0–0.89 mg chromium(III)/m^3); water-soluble chromium(VI) compounds (0.005–0.17 mg chromium(VI)/m^3); and acid-soluble/water-insoluble chromium compounds (including basic chromium sulfate), which may or may not entirely represent chromium(III) (0–0.47 mg chromium/m^3) (PHS 1953). Likewise, no effects on red blood cell counts, white blood cell counts, hemoglobin levels, or sedimentation rate were found in a case control study of 17 male manual metal arc stainless steel welders from six industries with mean occupational durations of 20 years (Littorin et al. 1984). The relationship between serum and urine chromium levels and blood hemoglobin was examined in workers exposed to chromium(III) at a tannery plant in Leon, Mexico (Kornhauser et al. 2002). Groups of workers were classified as unexposed (control; n=11), moderately

exposed (n=14) or highly exposed (n=11) based on job type; exposure levels were not reported. Blood chromium levels of 0.13, 0.25, and 0.39 μg/L and urine chromium levels of 1.35, 1.43, and 1.71 μg/L were observed in the control, moderate, and high exposure groups, respectively; statistically significant differences were observed between the control group and both chromium groups for blood chromium and between the control and the high exposure groups for urine chromium. An inverse relationship was observed between urine chromium and blood hemoglobin (r=-0.530), serum chromium and urine iron (r=-0.375) and the chromium/iron ratio in urine and hemoglobin (r=-0.669; <0.05). Results indicate a potential effect of chromium(III) exposure on hemoglobin; however, due to small group size, definitive conclusions cannot be made. No hematological disorders were found among 106 workers in a chromium(III) producing plant where workroom levels were ≤1.99 mg chromium(III)/m^3 as chromium(III) oxide and chromium(III) sulfate (Korallus et al. 1974a).

Results from hematological evaluations in rats yielded conflicting results. Hematological effects were observed in male Sprague-Dawley rats exposed to chromium trioxide mist for 90 days; changes included significant decreases in hematocrit (at 0.23 and 1.15, but not 0.49 mg chromium(VI)/m^3), hemoglobin (at 0.49 and 1.15 mg chromium(VI)/m^3) and erythrocyte count (at 1.15 mg chromium(VI)/m^3) (Kim et al. 2004). Hematological evaluations of rats exposed to sodium dichromate at 0.025–0.2 mg chromium(VI)/m^3 for 28 or 90 days or 0.1 mg chromium(VI)/m^3 for 18 months were unremarkable (Glaser et al. 1985, 1986, 1988). However, increased white blood cell counts were found in rats exposed to ≥0.1 mg chromium(VI)/m^3 as sodium dichromate for 30 days and at ≥0.05 mg chromium(VI)/m^3 for 90 days. The white blood cell counts were not increased 30 days postexposure (Glaser et al. 1990). Rats exposed to 0.1 mg chromium/m^3 as a 3:2 mixture of chromium(VI) trioxide and chromium(III) oxide for 18 months had increased red and white blood cell counts, hemoglobin content, and hematocrit (Glaser et al. 1986, 1988).

No changes in hematological parameters were observed in rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years (Lee et al. 1989).

In male and female CDF rats exposed to insoluble chromic oxide or soluble basic chromium sulfate by nose-only inhalation at 3, 10, or 30 mg chromium(III)/m^3 for 6 hours/day, 5 days/week for 13 weeks, no adverse effects on hematological parameters were observed (Derelanko et al. 1999).

Musculoskeletal Effects. No musculoskeletal effects have been reported in either humans or animals after inhalation exposure to chromium.

Hepatic Effects. Chromium(VI) has been reported to cause severe liver effects in four of five workers exposed to chromium trioxide in the chrome plating industry. Derangement of the cells in the liver, necrosis, lymphocytic and histiocytic infiltration, and increases in Kupffer cells were reported. Abnormalities in tests for hepatic dysfunction included increases in sulfobromophthalein retention, gamma globulin, icterus, cephalin cholesterol flocculation, and thymol turbidity (Pascale et al. 1952). In a cohort of 4,227 workers involved in production of stainless steel from 1968 to 1984, excess deaths were observed from cirrhosis of the liver compared to expected deaths (O/E=55/31.6) based on national rates and matched for age, sex, and calendar time having an SMR of 174 with confidence limits of 131–226 (Moulin et al. 1993). No measurements of exposure were provided. Based on limited information, however, the production of chromium compounds does not appear to be associated with liver effects. As part of a mortality and morbidity study of workers engaged in the manufacture of chromium(VI) compounds (84%) and chromium(III) compounds (16%) derived from chromium(VI) in Japan, 94 workers who had been exposed for 1–28 years were given a complete series of liver function tests 3 years after exposure ended. All values were within normal limits (Satoh et al. 1981). In a survey of a facility engaged in chromate production in Italy, where exposure concentrations were ≥0.01 mg chromium(VI)/m^3, 15 of 65 men who worked in the production of dichromate and chromium trioxide for at least 1 year had hepatobiliary disorders. When the workers were given liver function tests, slight impairment was found in a few cases. These disorders could have been due to a variety of factors, especially heavy alcohol use (Sassi 1956). No indication was found that exposure to chromium(III) resulted in liver disorders in workers employed in two factories that produced chromium(III) oxide or chromium(III) sulfate (Korallus et al. 1974b).

The hepatic effects observed in animals after inhalation exposure to chromium or its compounds were minimal and not considered to be adverse. Rats exposed to as much as 0.4 mg chromium(VI)/m^3 as sodium dichromate for ≤90 days did not have increased serum levels of alanine aminotransferase or alkaline phosphatase, cholesterol, creatinine, urea, or bilirubin (Glaser et al. 1990). Triglycerides and phospholipids were increased only in the 0.2 mg chromium(VI)/m^3 group exposed for 90 days (Glaser et al. 1985). No histopathological changes to the liver were observed in male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide (Kim et al. 2004) or in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months, (Derelanko et al. 1999). Chronic exposure of rats to 0.1 mg chromium(VI)/m^3 as sodium dichromate, to 0.1 mg total chromium/m^3 as a 3:2 mixture of chromium(VI) trioxide and chromium(III) oxide, or to

15.5 mg chromium(IV)/m^3 as chromium dioxide did not cause adverse hepatic effects as assessed by histological examination and liver function tests (Glaser et al. 1986, 1988; Lee et al. 1989).

Renal Effects. No increases in genital/urinary disease were evident in a cohort of 3,408 workers from four former facilities that produced chromium compounds from chromite ore in northern New Jersey sometime between 1937 and 1971. The proportionate mortality ratios for white and black men were 71 (40–117) and 47 (15–111), respectively. Exposure durations ranged from <1 to >20 years (Rosenman and Stanbury 1996).

Renal function has been studied in workers engaged in chromate and dichromate production, in chrome platers, in stainless steel welders, in workers employed in ferrochromium production, in boilermakers, and in workers in an alloy steel plant. Workers exposed to chromium(VI) compounds in a chromate production plant were found to have higher levels of a brush border protein antigen and retinol binding protein in the urine compared with controls (Mutti et al. 1985a). A similar study was conducted in 43 male workers in the chromate and dichromate production industry, where occupational exposures were between 0.05 and 1.0 mg chromium(VI)/m^3 as chromium trioxide, and mean employment duration was 7 years. Workers with >15 μg chromium/g creatinine in the urine had increased levels of retinol binding protein and tubular antigens in the urine (Franchini and Mutti 1988). These investigators believe that the presence of low molecular weight proteins like retinol binding protein or antigens in the urine are believed to be early indicators of kidney damage. In an extensive survey to determine the health status of chromate workers in seven U.S. chromate production plants, analysis of the urine revealed a higher frequency of white blood cell and red blood cell casts than is usually found in an industrial population (statistical significance not reported). Various manufacturing processes in the plants resulted in exposure of workers to chromite ore (mean time-weighted concentration of 0–0.89 mg chromium(III)/m^3); water-soluble chromium(VI) compounds (0.005–0.17 mg chromium(VI)/m^3); and acid-soluble/water-insoluble chromium compounds (including basic chromium sulfate), which may or may not entirely represent chromium(III) (0–0.47 mg chromium/m^3) (PHS 1953).

Some studies of renal function in chromate production workers found negative or equivocal results. In a survey of a facility engaged in chromate production in Italy, where exposure concentrations were ≥0.01 mg chromium(VI)/m^3, results of periodic urinalyses of workers who worked in the production of dichromate and chromium trioxide for at least 1 year were generally unremarkable, with the exception of one case of occasional albuminuria and a few cases of slight urobilinuria (Sassi 1956). As part of a mortality and morbidity study of workers engaged in the manufacture of chromium(VI) compounds

(84%) and chromium(III) compounds (16%) derived from chromium(VI) in Japan, 94 workers who had been exposed for 1–28 years were given a complete series of kidney function tests (not further characterized) 3 years after exposure ended. All values were within normal limits (Satoh et al. 1981).

Studies of renal function in chrome platers, whose exposure is mainly to chromium(VI) compounds, have also yielded equivocal results. A positive dose-response for elevated urinary levels of β_2-microglobulin was found in chrome platers who were exposed to 0.004 mg chromium(VI)/m^3, measured by personal air samplers, for a mean of 5.3 years. However, since no increase in β_2-microglobulin levels was found in ex-chrome platers who had worked for at least 1 year in an old chrome plating plant from 1940 to 1968, this effect may be reversible (Lindberg and Vesterberg 1983b). Liu et al. (1998) similarly found significantly higher urinary β_2-microglobulin and N-acetyl-β-glucosaminidase levels in hard-chrome electroplaters exposed to 0.0042 mg chromium/m^3 for a mean of 5.8 years, as compared to aluminum anode-oxidation workers. The prevalence of elevated levels (higher than reference values) was significantly increased for N-acetyl-β-glucosaminidase, but not for β_2-microglobulin. In another study, comparison of results of renal function tests between chrome platers and construction workers revealed that the chrome platers had significantly ($p<0.001$) increased levels of urinary chromium and increased clearance of chromium, but decreased ($p<0.05$) levels of retinol binding protein. However, no differences were found for blood urea nitrogen, serum and urinary β_2-microglobulin, serum immunoglobulin, total protein in the urine, urinary albumin, N-acetyl-β-D-glucosamidase, β-galactosidase, or lysozyme (Verschoor et al. 1988).

Studies of renal function in stainless steel welders, whose exposure is mainly to chromium(VI) compounds, were negative. Stainless steel welders had significantly increased ($p<0.001$) levels of urinary chromium, increased clearance of chromium, and increased serum creatinine compared with controls, but no differences were found in the levels of retinol binding protein, β_2-microglobulin, or other indices of kidney damage (Verschoor et al. 1988). Similar negative results were found in another group of stainless steel welders (Littorin et al. 1984).

Occupational exposure to chromium(III) or chromium(0) does not appear to be associated with renal effects. No renal impairment based on urinary albumin, retinol binding protein, and renal tubular antigens was found in 236 workers employed in the ferrochromium production industry where ferrochromite is reduced with coke, bauxite, and quartzite. The mean airborne concentration of chromium in various sample locations was 0.075 mg chromium(III)/m^3; chromium(VI) was below the detection limit of 0.001 mg chromium(VI)/m^3 at all locations (Foa et al. 1988). Workers employed in an alloy steel plant

with a mean exposure of 7 years to metallic chromium at 0.61 mg chromium(0)/m^3 and to other metals had normal urinary levels of total protein and β_2-microglobulin, enzyme activities of alanine-aminopeptidase, N-acetyl-β-D-glucosaminidase, gammaglutamyl-transpeptidase, and β-galactosidase (Triebig et al. 1987). In boilermakers exposed to chromium(0), no increase in urinary levels of chromium, and no differences in the levels of retinol binding protein, β_2-microglobulin, or other indices of renal toxicity were found (Verschoor et al. 1988).

In a group of 30 men and 25 women who were lifetime residents of an area in northern New Jersey contaminated with chromium landfill, signs of preclinical renal damage were assessed by examining the urinary levels of four proteins, intestinal alkaline phosphatase, tissue nonspecific alkaline phosphatase, N-acetyl-β-D-glucosaminidase, and microalbumin (Wedeen et al. 1996). The mean urinary chromium concentrations were 0.2±0.1 μg/g creatinine for the women and 0.3 μg/g creatinine for the men. None of the four proteins exceeded normal urinary levels in either men or women. The authors concluded that long-term environmental exposure to chromium dust did not lead to tubular proteinurea or signs of preclinical renal damage.

Exposure of rats to sodium dichromate at ≤0.4 mg chromium(VI)/m^3 for ≤90 days did not cause abnormalities, as indicated by histopathological examination of the kidneys. Serum levels of creatinine and urea and urine levels of protein were also normal (Glaser et al. 1985, 1990). No changes in urinalysis parameters or histopathological changes to the kidneys were observed in male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide (Kim et al. 2004) and no histopathological lesions were observed in the kidneys of male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months (Derelanko et al. 1999). Furthermore, no renal effects were observed in rats exposed to 0.1 mg chromium/m^3 as sodium dichromate (chromium(VI)) or as a 3:2 mixture of chromium(VI) trioxide and chromium(III) oxide for 18 months, based on histological examination of the kidneys, urinalysis, and blood chemistry (Glaser et al. 1986, 1988). Rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years showed no histological evidence of kidney damage or impairment of kidney function, as measured by routine urinalysis. Serum levels of blood urea nitrogen, creatinine, and bilirubin were also normal (Lee et al. 1989).

Endocrine Effects. Increased serum amylase activity (a marker for pancreatic function) was observed in a group of 50 chrome plating workers in Bangalore, India, compared to 50 workers with no history of chromium(VI) exposure. Employment duration of exposed workers ranged from 15 to 20 years; exposure levels were not reported (Kalahasthi et al. 2007). Serum amylase activity in exposed workers was

significantly correlated to urine chromium ($r=0.289$; $p<0.05$). No studies were located regarding endocrine effects in humans following inhalation exposure to chromium(III) compounds.

For intermediate-duration exposures, no histopathological changes to the endocrine tissues were observed in male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide (Kim et al. 2004) or in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months (Derelanko et al. 1999). Male rats exposed 22 hours/day for 18 months to 0.1 mg chromium(VI)/m^3 as sodium dichromate or exposed to a mixture of chromium(VI) and chromium(III) (0.06 mg chromium(VI)/m^3 plus 0.04 mg chromium(III)/m^3) as chromium(VI) trioxide and chromium(III) oxide did not result in any histopathological changes in adrenal glands (Glaser et al. 1986, 1988). Rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years showed no histopathological abnormalities in adrenals, pancreas, and thyroid glands (Lee et al. 1989).

Dermal Effects. Acute systemic and dermal allergic reactions have been observed in chromium-sensitive individuals exposed to chromium via inhalation as described in Sections 3.2.3.2 and 3.2.3.3.

No studies were located regarding systemic dermal effects in animals after inhalation exposure to chromium(VI) or chromium(III) compounds.

Ocular Effects. Effects on the eyes due to direct contact of the eyes with airborne mists, dusts, or aerosols or chromium compounds are described in Section 3.2.3.2. Medical records of 2,307 male workers (all nonsmokers) employed at a chromate production plant in Baltimore, Maryland between 1950 and 1974 were evaluated to determine the percentage of workers reporting clinical symptoms, mean time of employment to first diagnosis of symptoms, and mean exposure to chromium(VI) at the time of first diagnosis (exposure for each worker was the annual mean in the area of employment during the year of first diagnosis) (Gibb et al. 2000a). Conjunctivitis was reported on 20.0% of the study population, at a mean exposure level of 0.025 mg Cr(VI)/m^3 and a mean time-to-onset of 604 days.

Opthalmoscopic examination did not reveal any changes in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months (Derelanko et al. 1999).

Histopathologic examination of rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years revealed normal morphology of the ocular tissue (Lee et al. 1989).

Body Weight Effects. In a report of a case of acute exposure to "massive amounts" of chromium trioxide fumes, the patient became anorexic and lost 20–25 pounds during a 3-month period following exposure (Meyers 1950).

In rats exposed to an aerosol of sodium dichromate for 30 or 90 days or for 90 days followed by an additional 30 days of nonexposure, body weight gain was significantly decreased at 0.2 and 0.4 mg chromium(VI)/m^3 for 30 days ($p<0.001$), at 0.4 mg chromium(VI)/m^3 for 90 days ($p<0.05$), and at 0.2 ($p<0.01$) and 0.4 mg chromium(VI)/m^3 ($p<0.05$) in the recovery group (Glaser et al. 1990). There was no effect on body weight gain in rats exposed for 28 days to 0.2 mg/m^3 (Glaser et al. 1985) or for ≤18 months to 0.1 mg chromium(VI)/m^3 as sodium dichromate (Glaser et al. 1986, 1988, 1990) or 0.1 mg chromium(III and VI)/m^3 as a 3:2 mixture of chromium(VI) trioxide and chromium(III) oxide for 18 months (Glaser et al. 1986, 1988). Body weight was significantly decreased in male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide mist for 90 days (Kim et al. 2004) and in male, but not female, rats exposed to 10 mg chromium(III)/m^3 as chromic oxide for 13 weeks (Derelanko et al. 1999). However, exposure of male and female rats to 30 mg chromium(III)/m^3 as basic chromium sulfate for 13 weeks did not produce body weight changes (Derelanko et al. 1999). Similarly, there was no effect on body weight gain in rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years (Lee et al. 1989).

3.2.1.3 Immunological and Lymphoreticular Effects

Sensitization of workers, resulting in respiratory and dermal effects, has been reported in numerous occupational exposure studies. Although the route of exposure for initial sensitization in an occupational setting is most likely a combination of inhalation, oral, and dermal routes, information on the exposure levels producing sensitization by the inhaled route was not identified. Additional information on contact dermatitis in sensitized workers is provided in Section 3.2.3.3 (Dermal Exposure, Immunological and Lymphoreticular Effects).

Acute reactions have been observed in chromium sensitive individuals exposed to chromium via inhalation as noted in several individual case reports. A 29-year-old welder exposed to chromium vapors from chromium trioxide baths and to chromium and nickel fumes from steel welding for 10 years complained of frequent skin eruptions, dyspnea, and chest tightness. Chromium sensitivity in the individual was measured by a sequence of exposures, via nebulizer, to chromium(VI) as sodium chromate. Exposure to 0.029 mg chromium(VI)/mL as sodium chromate caused an anaphylactoid

reaction, characterized by dermatitis, facial angioedema, bronchospasms accompanied by a tripling of plasma histamine levels, and urticaria (Moller et al. 1986). Similar anaphylactoid reactions were observed in five individuals who had a history of contact dermatitis to chromium, after exposure, via nebulizer, to an aerosol containing 0.035 mg chromium(VI)/mL as potassium dichromate. Exposure resulted in decreased forced expiratory volume, facial erythema, nasopharyngeal pruritus, nasal blocking, cough, and wheezing (Olaguibel and Basomba 1989). Challenge tests with fumes from various stainless steel welding processes indicated that the asthma observed in two stainless steel welders was probably caused by chromium or nickel, rather than by irritant gases produced by the welding process (Keskinen et al. 1980). A 28-year-old construction worker developed work-related symptoms of asthma, which worsened during periods when he was working with (and sawing) corrugated fiber cement containing chromium. A skin patch test to chromium was negative. Asthmatic responses were elicited upon inhalation challenge with fiber cement dust or nebulized potassium chromate (Leroyer et al. 1998). A 40-year-old woman exposed to chromium and nickel in a metalworks company developed occupational asthma and tested positive to skin prick tests and bronchial challenge tests with potassium dichromate (Cruz et al. 2006). In four male workers (two electroplating workers, one welder, and one cement worker) with work-related symptoms of asthma, two tested positive to skin prick tests with potassium dichromate and nickel sulfate and all tested positive to bronchial challenge tests with potassium dichromate and nickel sulfate (Fernandez-Nieto et al. 2006). Chromium-induced asthma may occur in some sensitized individuals exposed to elevated concentrations of chromium in air, but the number of sensitized individuals is low and the number of potentially confounding variables in the chromium industry is high.

Concentrations of some lymphocyte subpopulations (CD4+ helper-inducer, CD5--CD19+ B, CD3--CD25+ activated B, and CD3--HLA-DR+ activated B and natural killer lymphocytes) were significantly reduced (about 30–50%) in a group of 15 men occupationally exposed to dust containing several compounds (including hexavalent chromium as lead chromate) in a plastics factory. Worker blood lead and urine chromium levels were significantly higher than those of 15 controls not known to be occupationally exposed to toxic agents. Serum chromium concentrations and serum immunoglobulins IgA, IgG, and IgM were not significantly different between the two groups (Boscolo et al. 1997). The immunological effects of chromium were evaluated in a small group tannery workers (n=20) in Italy, compared to a matched group of unexposed controls (n=24) (Mignini et al. 2004). Exposure of individual workers was not reported, but monitoring of 20 factories with participating workers reported TWA concentrations of 0.09–0.10 mg total chromium/m^3 and 0.001–0.002 mg chromium(VI)/m^3. The mean time of employment of the exposed group was 5.8 years. Urine chromium excretion was significantly

increased in workers, although no increased in plasma chromium was observed, compared to controls. In workers, proliferative response of peripheral blood mononucleocytes (PBMC) in response to concavalin A was increased approximately 24% compared to controls; no difference between workers and controls were observed for the percent distribution of lymphocyte subsets (e.g., T lymphocytes, T helper lymphocytes, T cytotoxic lymphocytes, B lymphocytes, and natural killer cells).

Immunological effects of exposure to chromic acid were evaluated in 46 electroplating workers in Taiwan (Kuo and Wu 2002). The entire group was employed for an average of 6.1 years. Workers were divided into low (n=19), moderate (n=17), and high (n=10) subgroups based on mean urine chromium excretion of <1.13, 1.14–6.40, and >6.40 μg chromium/g creatinine, respectively. Airborne chromium was measured by personal samplers for all study participants for the duration of one 8-hour shift (data not reported); however, no information was reported on individual or group exposures over the time of employment. A negative correlation was observed between urine chromium and B cell percentage and a positive correlation was observed between urine chromium and blood IL-8 concentration. The study authors report that smoking was an important factor for lymphocyte subsets; thus, interpretation of these results is limited by confounding factors.

An animal study was designed to examine the immunotoxic effects of soluble and insoluble hexavalent chromium agents released during welding (Cohen et al. 1998). Rats exposed to atmospheres containing soluble potassium chromate at 0.36 mg chromium(VI)/m^3 for 5 hours/day, 5 days/week for 2 or 4 weeks had significantly increased levels of neutrophils and monocytes and decreased alveolar macrophages in bronchoalveolar lavage than air-exposed controls. Significantly increased levels of total recoverable cells were noted at 2 (but not 4) weeks of exposure. In contrast, no alterations in the types of cells recovered from the bronchoalveolar lavage fluid were observed in rats exposed to 0.36 mg chromium(VI)/m^3 as insoluble barium chromate, as compared to controls. However, the cell types recovered did differ from those recovered from rats exposed to soluble chromium. Changes seen in pulmonary macrophage functionality varied between the soluble and insoluble chromium(VI) exposure groups. The production of interleukin (IL)-1 and tumor necrosis factor (TNF)-α cytokines were reduced in the potassium chromate exposed rats; only TNF-α was decreased in the barium chromate rats. IL-6 levels were not significantly altered in either group. Barium chromate affected zymosan-inducible reactive oxygen intermediate formation and nitric oxide production to a greater degree than soluble chromium(VI). Insoluble chromium(VI) reduced the production of superoxide anion, hydrogen perodise, and nitric oxide; soluble chromium(VI) only reduced nitric oxide production.

Rats exposed to 0.025–0.2 mg chromium(VI)/m^3 as sodium dichromate for 28 or 90 days had increased spleen weights at ≥0.05 mg chromium(VI)/m^3 and increased response to sheep red blood cells at ≥0.025 mg chromium(VI)/m^3. In the 90-day study, serum immunoglobulin content was increased in the 0.05 and 0.1 mg chromium(VI)/m^3 groups but not in the 0.2 mg chromium(VI)/m^3 group. There was an increase in mitogen-stimulated T-cell response in the group exposed for 90 days to 0.2 mg chromium(VI)/m^3. Bronchial alveolar lavage fluid had an increased percentage of lymphocytes in the groups exposed to 0.025 and 0.05 mg chromium(VI)/m^3 and an increased percentage of granulocytes in the groups exposed to 0.05 mg chromium(VI)/m^3 for 28 days. The phagocytic activity of macrophages was increased in the 0.05 mg chromium(VI)/m^3 group. A higher number of macrophages in telophase was observed in the 0.025 and 0.05 mg chromium(VI)/m^3 groups. Bronchial alveolar lavage fluid from rats exposed for 90 days had an increased percentage of lymphocytes in the 0.025, 0.05, and 0.2 mg chromium(VI)/m^3 groups and an increased percentage of granulocytes and number of macrophages in the 0.05 mg chromium(VI)/m^3 groups. The phagocytic activity of the macrophages was increased in the 0.025 mg and 0.05 mg chromium(VI)/m^3 groups and decreased in the 0.2 mg chromium(VI)/m^3 group. A greater number of macrophages in telophase and an increase in their diameter were observed in the 0.025, 0.05, and 0.2 mg chromium(VI)/m^3 groups (Glaser et al. 1985).

Low-level exposure to sodium dichromate seems to stimulate the humoral immune system (as indicated by the significant increase in total immunoglobin levels); exposure to 0.2 mg chromium(VI)/m^3 ceases to stimulate the humoral immune system (significant decreases in total immunoglobin levels) but still may have effects on the T lymphocytes. The depression in macrophage cell count and phagocytic activities correlated with a 4-fold lower rate of lung clearance for inhaled iron oxide in the 0.2 mg chromium(VI)/m^3 group (Glaser et al. 1985).

Intermediate-duration exposure of rats to inhaled chromium(III) compounds produces histopathological alterations to respiratory lymph nodes and tissues. In male and female CDF rats, exposure to 3, 10, and 30 mg chromium(III)/m^3 as soluble basic chromium sulfate for 13 weeks resulted in histiocytic cellular infiltration and hyperplasia of peribronchial lymphoid tissue and mediastinal lymph nodes; lymph node enlargement was also observed on necropsy (Derelanko et al. 1999). Following a 13-week recovery period, enlargement, histiocytosis, and hyperplasia of the mediastinal lymph node was observed in rats exposed to 3, 10, and 30 chromium(III)/m^3 as basic chromium sulfate. Hyperplasia of the mediastinal lymph node was observed in male and female CDF rats exposed to chromium oxide at concentrations of 3, 10, and 30 mg chromium(III)/m^3 for 13 weeks (Derelanko et al. 1999). Following a 13-week recovery

period, black pigment (trace-to-mild) in peribronchial lymphoid tissue and mediastinal lymph nodes was found in all treatment groups.

The LOAELs for immunological effects in rats are recorded in Table 3-1 and plotted in Figure 3-1 for chromium(VI) and recorded in Table 3-2 and plotted in Figure 3-2 for chromium(III).

3.2.1.4 Neurological Effects

In a chrome plating plant where poor exhaust resulted in excessively high concentrations of chromium trioxide fumes, workers experienced symptoms of dizziness, headache, and weakness when they were working over the chromate tanks (Lieberman 1941). Such poor working conditions are unlikely to still occur in the United States because improvements in industrial hygiene have been made over the years. Results of olfactory perceptions tests conducted in workers employed at chromium plating factories in An-San Korea (mean employment duration of 7.9 years) indicate that olfactory recognition thresholds were significantly higher in exposed workers compared to controls (Kitamura et al. 2003). Air concentrations of chromium(VI) ranged from 0.005 to 0.03 mg chromium(VI)/m^3 and of chromium(III) ranged from 0.005 to 0.06 mg chromium(III)/m^3. Although the cause of this change was not determined, the study authors suggest that chromium may directly affect the olfactory nerve.

No increases in vascular lesions in the central nervous system were evident in a cohort of 3,408 workers from four former facilities that produced chromium compounds from chromite ore in northern New Jersey (Rosenman and Stanbury 1996). The proportionate mortality ratios for white and black men were 78 (61–98) and 68 (44–101), respectively. The subjects were known to have worked in the four facilities sometime between 1937 and 1971 when the last facility closed. Exposure durations ranged from <1 to >20 years.

No information was located regarding neurological effects in humans or animals after inhalation exposure to chromium(III) compounds or in animals after inhalation exposure to chromium(VI) compounds. No histopathological lesions were found in the brain of male Sprague-Dawley rats exposed to 1.15 mg chromium(VI)/m^3 as chromium trioxide for 3 months or in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months (Derelanko et al. 1999; Kim et al. 2004) or in the brain, spinal cord, or nerve tissues of rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years (Lee et al. 1989). No neurological or behavioral tests were conducted in these studies.

3.2.1.5 Reproductive Effects

Information regarding reproductive effects in humans after inhalation to chromium compounds is limited. Semen quality was evaluated in 61 workers in a chromium sulfate manufacturing plant in India (Kumar et al. 2005). Employment duration and chromium exposure levels were not reported. The study included a control group of 15 unexposed workers. Chromium blood levels in the exposed group were significantly increased compared to the control group. Although no effect was observed on semen volume, liquefaction time, or pH or on sperm viability, count, motility, or concentration, a significant increase was observed in the number of morphologically abnormal sperm in exposed workers. In the exposed group, 53% of subjects had less than 30% normal sperm; in the control group, only 10% of subject had <30% normal sperm. A significant positive correlation ($r=0.301$; $p=0.016$) was observed between blood chromium and the percentage of abnormal sperm in exposed workers. Sperm count and motility were significantly decreased by 47 and 15%, respectively in a group of 21 workers employed at a chrome plating plant in Henan, China, compared to age-matched, unexposed controls (Li et al. 2001). Serum follicle stimulating hormone (FSH) concentration was significantly increased by 204% and semen lactate dehydrogenase activity was significantly decreased by 30% in exposed compared to control workers, although no effect on serum luteinizing hormone (LH) concentration was observed. Serum chromium levels were 11% higher in the exposed workers compared to control; however, the increase was not statistically significant. Duration of employment for all study participants ranged from 1 to 15 years; no information on exposure levels or demographics of the exposed and control groups were reported.

The effect of chromium(VI) on the course of pregnancy and childbirth was studied in women employees at a dichromate manufacturing facility in Russia. Complications during pregnancy and childbirth (not further described) were reported in 20 of 26 exposed women who had high levels of chromium in blood and urine, compared with 6 of 20 women in the control group. Toxicosis (not further described) was reported in 12 exposed women and 4 controls. Postnatal hemorrhage occurred in four exposed and two control women (Shmitova 1980). Similar results were reported in a more extensive study of 407 women who worked at a factory producing chromium compounds (not otherwise specified) compared with 323 controls. The frequency of birth complications was 71.4% in a subgroup of highly exposed women, 77.4% in a subgroup of women with a lower level of exposure, and 44.2% in controls. Toxicosis in the first half of pregnancy occurred in 35.1% of the high exposure group, 33.3% of the low exposure group, and 13.6% of the controls. The frequency of postnatal hemorrhage was 19.0% for the high exposure group and 5.2% in controls (Shmitova 1978). Because these studies were generally of poor quality and

the results were poorly reported, no conclusions can be made regarding the potential for chromium to produce reproductive effects in humans.

The occurrence of spontaneous abortion among 2,520 pregnancies of spouses of 1,715 married Danish metal workers exposed to hexavalent chromium from 1977 through 1987 were examined (Hjollund et al. 1995). Occupational histories were collected from questionnaires and information on spontaneous abortion, live births, and induced abortion was obtained from national medical registers. The number of spontaneous abortions was not increased for pregnant women whose spouses worked in the stainless steel welding industry when compared to controls (odds ratio 0.78, 95% confidence interval [CI] 0.55–1.1). The authors believed that the risk estimate was robust enough that factors such as maternal age and parity and smoking and alcohol consumptions were not confounders. There was no association found in spontaneous abortions in women whose husbands were in the cohort subpopulations who were mild steel welders and metal-arc stainless steel welders, which would lead to higher exposures to welding fumes (workplace chromium exposures not provided). This more recent study does not corroborate earlier findings (Bonde et al. 1992) that showed that wives of stainless steel welders were at higher risk of spontaneous abortions. The current study was based on abortions recorded in a hospital register, while the earlier study was based on self-reporting data. The latter study probably included more early abortions and was biased because the job exposure of male metal workers is apparently modified by the outcome of their partners' first pregnancy.

Histopathological examination of the testes of rats exposed to 0.2 mg chromium(VI)/m^3 as sodium dichromate for 28 or 90 days (Glaser et al. 1985), to 0.1 mg chromium(VI)/m^3 as sodium dichromate for 18 months, or to 0.1 mg chromium/m^3 as a 3:2 mixture of chromium(VI) trioxide and chromium(III) oxide for 18 months (Glaser et al. 1986, 1988) revealed no abnormalities. For intermediate-duration exposures to chromium(III) compounds, no histopathological changes to the reproductive tissues in male and female CDF rats exposed to 30 mg chromium(III)/m^3 as chromic oxide or basic chromium sulfate for 3 months; treatment also had no effect on sperm count, motility, or morphology (Derelanko et al. 1999). No histopathological lesions were observed in the prostate, seminal vesicle, testes, or epididymis of male rats or in the uterus, mammary gland, or ovaries of female rats exposed to 15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years (Lee et al. 1989).

The NOAELs for reproductive effects in rats are recorded in Table 3-1 and plotted in Figure 3-1 for chromium(VI) and recorded in Table 3-2 and plotted in Figure 3-2 for chromium(III).

3.2.1.6 Developmental Effects

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

3.2.1.7 Cancer

Occupational exposure to chromium(VI) compounds in various industries has been associated with increased risk of respiratory system cancers, primarily bronchogenic and nasal. Among the industries investigated in retrospective mortality studies are chromate production, chromate pigment production and use, chrome plating, stainless steel welding, ferrochromium alloy production, and leather tanning. Compilations and discussion of many of these studies can be found in reviews of the subject (Goldbohm et al. 2006; IARC 1990; Steenland et al. 1996). Studies of chromium workers have varied considerably in strength of design for determining cancer risks related to chromium exposure. The strongest designs have provided estimates of chromium(VI) (or exposure to other chromium species) for individual members of the cohorts, enabling application of dose-response analysis to estimate the contribution of chromium exposure to cancer risk. Studies that do not provide estimates of chromium exposure have relied on surrogate dose metrics (e.g., length of employment at job titles associated with chromium exposure) for exploring attribution of cancer risk to chromium exposure. However, these surrogate measures are often strongly correlated with exposures to other work place hazards, making conclusions regarding possible associations with chromium exposures more uncertain. Chromium dose-response relationships have been reported for chromate production workers, but not for other categories of chromium workers. In studies of chromate production workers, increased risk of respiratory tract cancers have been found in association with increased cumulative exposure to chromium(VI) and several estimates of excess lifetime risk attributed to chromium exposure have been reported. Studies of chrome platers, who were exposed to chromium(VI) and other carcinogenic chemicals, including nickel, have found significant elevations in lung cancer risk in association with surrogate indicators of chromium exposure, such as duration of employment at jobs in which exposure to chromium occurred; however, estimates of risk attributable to specifically to chromium exposure have not been reported. Results of studies in stainless steel welders exposed to chromium(VI) and other chemicals, and in ferrochromium alloy workers, who were exposed mainly to chromium(0) and chromium(III), but also to some chromium(VI), have been mixed and are inconclusive with respect to work-associated elevations in cancer rates. Studies in leather tanners, who are exposed to chromium(III), have not found elevated cancer rates.

Chromate Production. Numerous studies of cancer mortality among chromate production workers have been reported (Alderson et al. 1981; Bidstrup and Case 1956; Buckell and Harvey 1951; Crump et al. 2003; Davies et al. 1991; Enterline 1974; Gibb et al. 2000b; Korallus et al. 1982; Mancuso 1997a; Ohsaki et al. 1978; Park and Stayner 2006; Park et al. 2004; Pastides et al. 1994; PHS 1953; Rosenman and Stanbury 1996; Sassi 1956; Satoh et al. 1994; Taylor 1966). Collectively, these studies provide evidence for associations between lung cancer mortality and employment in chromate production, with risks declining with improved industrial hygiene. Less consistently, nasal cancers have been observed (Alderson et al. 1981; Rosenman and Stanbury 1996; Sassi 1956; Satoh et al. 1994). Evidence for associations between exposure to chromium and cancer is strongest for lung cancer mortality, which has been corroborated and quantified in numerous studies. A meta-analysis of 49 epidemiology studies based on 84 papers of cancer outcomes, primarily among chromium workers, found SMRs ranging from 112 to 279 for lung cancer, with and overall SMR of 141 (95% CI 135–147; Cole and Rodu 2005). When limited to high-quality studies controlled for smoking, the overall SMR for lung cancer was 112 (95% CI 104–119). SMRs for other forms of cancer from studies that controlled for confounders were not elevated. Several studies have attempted to derive dose-response relationships for this association (Crump et al. 2003; Gibb et al. 2000b; Mancuso 1997a; Park and Stayner 2006; Park et al. 2004). These studies are particularly important because they have included individual exposure estimates to chromium for each member of the cohort based on work place monitoring; dose-response modeling to ascertain the contribution of changing exposures to chromium to risk (in workers who were also exposed to other work-place hazards that could have contributed to cancer risk); and evaluation of the impacts of potential co-variables and confounders (e.g., age, birth cohort, and smoking) on chromium-associated risk.

Gibb et al. (2000b) examined lung cancer mortality in a cohort of chromate production workers (n=2,357, males) in Baltimore, Maryland, who were first hired during the period 1950–1974, with mortality followed through 1992. This cohort was the subject of numerous earlier studies, which found significantly increased lung cancer mortality (i.e., standard mortality ratios) among workers at the plant (Baetjer 1950b; Braver et al. 1985; Hayes et al. 1979; Hill and Ferguson 1979). In the Gibb et al. (2000b) study, cumulative exposures to chromium(VI) or chromium(III) (mg/m^3-year) were reconstructed for each member of the cohort from historical workplace air monitoring data and job title records (Gibb et al. 2000b). Lung cancer for the entire group had a relative risk of 1.80 (95% CI 1.49–2.14). Relative risk of lung cancer mortality (adjusted for smoking) increased by a factor to 1.38 (95% CI 1.20–1.63) in association with a 10-fold increase in cumulative exposure to chromium(VI). The analogous relative risk for cumulative exposure to chromium(III) was 1.32 (95% CI 1.15–1.51). Exposures to chromium(III) and chromium(VI) were highly correlated; therefore, discrimination of risks associated with either were

problematic. However, in a combined model that included cumulative exposure to both chromium species, relative risk for chromium(VI) exposure remained significant (1.66, p=0.045), whereas relative risk for chromium(III) was negative (-0.17, p=0.4). This outcome suggests that exposure to chromium(VI), rather than chromium(III), was the dominant (if not sole) contributor to lung cancer risk (after adjustments for smoking). Park et al. (2004) reanalyzed the data for the Baltimore, Maryland cohort using a variety of dose-response models. In the preferred model (linear with cumulative chromium exposure and log-linear for age, smoking, race), cancer rate ratio for a 45-year cumulative exposure to 1 mg/m^3-year of chromium(VI) was estimated to be 2.44 (95% CI 1.54–3.83). This corresponded to an excess lifetime risk unit risk (i.e., additional lifetime risk from occupational exposure to 1 μg CrO_3/m^3 or 0.52 μg Cr(VI)/m^3) of 0.003 (95% CI 0.001–0.006) or to 100 μg chromium(VI)/m^3 of 0.255 (95% CI 0.109–0.416). Subsequent analyses conducted by Park and Stayner (2006) attempted to estimate possible thresholds for increasing lung cancer risk. This analysis was able to exclude possible thresholds in excess of 16 μg/m^3 chromium(VI) or 0.4 mg/m^3-year cumulative exposure to chromium(VI).

Several studies have examined cancer mortality in a cohort of chromate production workers in Painesville, Ohio, and have found increased lung cancer mortality (e.g., SMRs) among workers at the plant (Crump et al. 2003; Luippold et al. 2003; Mancuso 1997a; Mancuso and Hueper 1951). Mancuso (1997a) reconstructed cumulative exposure histories of individual members of the cohort (n=332), hired during the period 1931–1937 and followed through 1993. The exposure estimations were based on historical workplace air monitoring data for soluble and insoluble chromium and job title records. Age-adjusted death rates from lung cancer were estimated for cumulative exposure strata, and increased with increasing cumulative exposure to total chromium, insoluble chromium, and soluble chromium (a dose response model was not reported). The highest rates were observed in soluble chromium strata >4 mg/m^3-years (2,848 per 100,000). Death rates were not adjusted for smoking, which would have been a major contributor to lung cancer death rates in the cohort. Although the study discriminated exposures to soluble and insoluble chromium, these classifications are not adequate surrogates for exposures to trivalent or hexavalent chromium (Kimbrough et al. 1999; Mundt and Dell 1997); therefore, the study cannot attribute risk specifically to either species. More recent studies of this cohort have attempted to reconstruct individual exposure histories to chromium(VI), based on species-specific air monitoring data, and have attempted to quantify the potential contribution of smoking to lung cancer risk (Crump et al. 2003; Luippold et al. 2003). These studies included workers (n=482) hired after 1940 and followed through 1997. Increasing lung cancer risk was significantly associated with increasing cumulative exposure to chromium(VI). Relative risk for lung cancer mortality was estimated to be 0.794 per mg/m^3-year (90% CI 0.518–1.120). The analogous additive risk was 0.00161 per mg/m^3-year per person year

(90% CI 0.00107–0.00225). These estimates correspond to unit risks (i.e., additional lifetime risk from occupational exposure to 1 μg/m^3) of 0.00205 (90% CI 0.00134–0.00291), based on the relative risk Poison model, and 0.00216 (90% CI 0.00143–0.00302), based on the additional risk Poison model. Risk estimates were not appreciably sensitive to birth cohort or to smoking designation (for the 41% of the cohort that could be classified). The latter outcome suggests that smoking did not have a substantial effect on chromium(VI) associated lung cancer risk (i.e., smoking and chromium appeared to contribute independently to cancer risk).

A meta-analysis of the Crump et al. (2003); Gibb et al. (2000b), and Mancuso (1997a) studies has also been reported (Goldbohm et al. 2006). Excess lifetime risk of lung cancers was estimated from a life table analysis (using Dutch population vital statistics) and estimates of relative risk from each study, or in the case of Mancuso (1997a), estimated in the meta-analysis (approximately 0.0015 per mg/m^3-year). Estimates of excess lifetime risks (deaths attributed to a 40-year occupational exposure to chromium(VI) at 1 μg/m^3, for survival up to age 80 years) were 0.0025, 0.0048, and 0.0133, based on Crump et al. (2003), Mancuso et al. (1997a), and Gibb et al. (2000b), respectively.

In conclusion, despite limitations of some studies, occupational exposure to chromium(VI) in the chromate production industry is associated with increased risk of respiratory cancer. Estimates of excess lifetime occupational risks range from 0.002 to 0.005 per μg/m^3 of chromium(VI). Changes in production process and industrial hygiene appear to have reduced overall risk over the past 30–40 years.

Chromate Pigments Production and Use. Studies of workers engaged in the production of chromate pigments provide evidence for increased risk of lung cancer associated with employment in work areas where exposure to chromium compounds occurred. However, the contribution of chromium exposure to cancer risk in these cohorts remains uncertain for several reasons: (1) members of the cohorts experienced exposures to a variety of chemicals that may have contributed to cancer (e.g., nickel); (2) exposures of the individual cohort members to chromium were not quantified or subjected to exposure-response analysis; and (3) dose metrics used in dose-response analysis were measures of employment duration, which are highly correlated with exposures to chemical hazards other than chromium. Nevertheless, these studies have found elevated lung cancer rates in chromium pigment workers in comparison to reference populations (e.g., SMRs) and, in some studies, increased lung cancer rates in association with increased potential (e.g., job type, employment duration) for exposure to chromium (Dalager et al. 1980; Davies 1979, 1984; Franchini et al. 1983; Frentzel-Beyme 1983;

Haguenoer et al. 1981; Hayes et al. 1989; Langård and Norseth 1975; Langård and Vigander 1983; Sheffet et al. 1982).

Chrome Plating. Studies of chrome platers provide evidence for increased risk of lung cancer associated with employment in work areas where exposure to chromium compounds occurred. However, the contribution of chromium exposure to cancer risk in these cohorts remains uncertain for several reasons: members of the cohorts experienced exposures to a variety of chemicals that may have contributed to cancer (e.g., nickel, sulfuric acid); (2) exposures of the individual cohort members to chromium were not quantified or subjected to exposure-response analysis; and (3) dose metrics used in dose-response analysis were measures of employment duration, which are highly correlated with exposures to chemical hazards other than chromium. Nevertheless, these studies have founds elevated lung cancer rates in chrome bath workers in comparison to reference populations (e.g., standard mortality ratios) who were exposed primarily to soluble chromium(VI) (e.g., chromic acid mists) and, in some studies, increased lung cancer rates in association with increased potential (e.g., job type, employment duration) for exposure to chromium (Dalager et al. 1980; Guillemin and Berode 1978; Hanslian et al. 1967; Okubo and Tsuchiya 1977, 1979; Royle 1975a; Silverstein et al. 1981; Sorahan et al. 1987, 1998; Takahashi and Okubo 1990).

Sorahan et al. (1998) examined lung cancer risks in a cohort of nickel/chrome platters (n=1,762, hired during the period 1946–1975 with mortality follow-up through 1995). The same cohort was studied by Royle (1975a). Significant excess risks of lung cancer were observed among males and females working in the chrome bath area for <1 year (SMR=172; 95% CI 112–277; $p<0.05$) or >5 years (SMR=320; 95% CI 128–658; $p<0.001$), females working in the chrome bath area for <1 year (SMR=245; 95% CI 118–451; $p<0.5$), males starting chrome work in the period of 1951–1955 (SMR=210; 95% CI 132–317; $p<0.01$), and in male chrome workers 10–19 years after first chrome work (SMR=203; 95% CI 121–321; $p<0.01$). A significant ($p<0.01$) positive trend for lung cancer mortality and duration of exposure was found for the male chrome bath workers, but not for the female workers. Lung cancer mortality risks were also examined using an internal standard approach, in which mortality in chrome workers was compared to mortality in workers without chromium exposure. After adjusting for sex, age, calendar period, year of starting chrome work, period from first chrome work, and employment status, a significant positive trend ($p<0.05$) between duration of chrome bath work and lung cancer mortality risk was found.

Stainless Steel Welding. Workers in the stainless steel welding industry are exposed to chromium(VI) compounds, as well as other chemical hazards that could contribute to cancer (e.g., nickel); however, results of studies of cancer mortality in these populations have been mixed. Some studies have found

increased cancer mortality rates among workers; however, examinations of possible associations with exposures to chromium have not been reported. A study of 1,221 stainless steel welders in the former Federal Republic of Germany found no increased risk of lung cancer or any other specific type of malignancy compared with 1,694 workers involved with mechanical processing (not exposed to airborne welding fumes) or with the general population of the former Federal Republic of Germany (Becker et al. 1985). A follow-up study (Becker 1999) which extended the observation period to 1995, found similar results for lung (includes bronchus and trachea) cancer (SMR=121.5, 95% CI 80.7–175.6). An excess risk of pleura mesothelioma was observed (SMR=1,179.9; 95% CI=473.1–2430.5); however, this was attributed to asbestos exposure. A study of 234 workers from eight companies in Sweden, who had welded stainless steel for at least 5 years during the period of 1950–1965 and followed until 1984, found five deaths from pulmonary tumors, compared with two expected (SMR=249), based on the national rates for Sweden. The excess was not statistically significant. However, when the incidence of lung cancer in the stainless steel welders was compared with an internal reference group, a significant difference was found after stratification for age. The average concentration of chromium(VI) in workroom air from stainless steel welding, determined in 1975, was reported as 0.11 mg/m^3 (Sjogren et al. 1987). The cohort in this study was small, and stainless welders were also exposed to nickel fumes. Smoking was probably not a confounding factor in the comparisons with the internal reference group.

In a study of the mortality patterns in a cohort of 4,227 workers involved in the production of stainless steel from 1968 to 1984, information was collected from individual job histories, and smoking habits were obtained from interviews with workers still active during the data collection (Moulin et al. 1993). The observed number of deaths was compared to expected deaths based on national rates and matched for age, sex, and calendar time. No significant excess risk of lung cancer was noted among workers employed in melting and casting stainless steel (SMR=104). However, there was a significant excess among stainless steel foundry workers (SMR=229). The SMR increased for workers with length of employment over 30 years to 334 (119–705). No measurements of exposure were provided.

Ferrochromium Production. Workers in the ferrochromium alloy industry are exposed to chromium(III) and chromium(VI) compounds, as well as other chemical hazards that could contribute to cancer; however, results of studies of cancer mortality in these populations have been mixed. No significant increase in the incidence of lung cancer was found among 1,876 employees who worked in a ferrochromium plant in Sweden for at least 1 year from 1930 to 1975 compared with the expected rates for the county in which the factory was located. The workers had been exposed mainly to metallic chromium and chromium(III), but chromium(VI) was also present. The estimated levels ranged from 0 to

2.5 mg chromium(0) and chromium(III)/m^3 and from 0 to 0.25 mg chromium(VI)/m^3 (Axelsson et al. 1980). An excess of lung cancer was found in a study of 325 male workers employed for >1 year in a ferrochromium producing factory in Norway between 1928 and 1977 (Langård et al. 1980), and whose employment began before 1960 (SMR=850, p=0.026); however, in a follow-up of this cohort (n=379, hired before 1965 and followed through 1985), the SMR for lung cancer was not significant (SMR=154; Langård et al. 1990). Workroom monitoring in 1975 indicated that the ferrochromium furnace operators worked in an atmosphere with 0.04–0.29 mg total chromium/m^3, with 11–33% of the total chromium as chromium(VI) (Langård et al. 1980).

An ecological study examined the distribution of lung cancer cases in Dolný Kubin in the Slovak Republic where ferrochromium production facility was located. Cases were stratified into three groups (males): ferrochomium workers (n=59), workers (n=106) thought not to have been exposed to chromium, and residents (n=409) who were not thought to have had appreciable exposure to chromium. Lung cancer rates were higher in the chromium workers (320 per 1,000 per year, 95% CI 318–323) compared to workers (112, 95% CI 109–113) and residents (79, 95% CI 76–80) who were not thought to have been exposed to chromium (relative risk=4.04 for chromium workers compared to residents). Mean work shift air concentrations in the smelter were 0.03–0.19 mg/m^3 for total chromium and 0.018–0.03 mg/m^3 for chromium(VI). Theses estimates were not adjusted for smoking or other potential co-variables that might have contributed to cancer rates in the chromium workers.

Leather Tanning. Studies of workers in tanneries, where exposure is mainly to chromium(III), in the United States (0.002–0.054 mg total chromium/m^3) (Stern et al. 1987), the United Kingdom (no concentration specified) (Pippard et al. 1985), and the Federal Republic of Germany (no concentration specified) (Korallus et al. 1974a) reported no association between exposure to chromium(III) and excess risk of cancer.

Environmental Exposure. In addition to the occupational studies, a retrospective environmental epidemiology study was conducted of 810 lung cancer deaths in residents of a county in Sweden where two ferrochromium alloy industries are located. No indication was found that residence near these industries is associated with an increased risk of lung cancer (Axelsson and Rylander 1980).

A retrospective mortality study conducted on a population that resided in a polluted area near an alloy plant that smelted chromium in the People's Republic of China found increased incidences of lung and stomach cancer. The alloy plant began smelting chromium in 1961 and began regular production in 1965,

at which time sewage containing chromium(VI) dramatically increased. The population was followed from 1970 to 1978. The size of the population was not reported. The adjusted mortality rates of the exposed population ranged from 71.89 to 92.66 per 100,000, compared with 65.4 per 100,000 in the general population of the district. The adjusted mortality rates for lung cancer ranged from 13.17 to 21.39 per 100,000 compared with 11.21 per 100,000 in the general population. The adjusted mortality rates for stomach cancer ranged from 27.67 to 55.17 per 100,000 and were reported to be higher than the average rate for the whole district (control rates not reported). The higher cancer rates were found for those who lived closer to the dump site (Zhang and Li 1987). Attempts to abate the pollution from chromium(VI) introduced in 1967 also resulted in additional pollution from sulfate and chloride compounds. It was not possible to estimate exposure levels based on the description of the pollution process. Exposure of this population was mainly due to chromium(VI) in drinking water, although air exposure cannot be ruled out.

The studies in workers exposed to chromium compounds clearly indicate that occupational exposure to chromium(VI) is associated with an increased risk of respiratory cancer. Using data from the Mancuso (1975) study and a dose-response model that is linear at low doses, EPA (1984a) derived a unit risk estimate of $1.2x10^{-2}$ for exposure to air containing 1 μg chromium(VI)/m^3 (or potency of $1.2x10^{-2}$ [μg/m^3]$^{-1}$) (IRIS 2008).

Chronic inhalation studies provide evidence that chromium(VI) is carcinogenic in animals. Mice exposed to 4.3 mg chromium(VI)/m^3 as calcium chromate had a 2.8-fold greater incidence of lung tumors, compared to controls (Nettesheim et al. 1971). Lung tumors were observed in 3/19 rats exposed to 0.1 mg chromium(VI)/m^3 as sodium dichromate for 18 months, followed by 12 months of observation. The tumors included two adenomas and one adenocarcinoma. No lung tumors were observed in 37 controls or the rats exposed to ≤0.05 mg chromium(VI)/m^3 (Glaser et al. 1986, 1988). The increased incidence of lung tumors in the treated rats was significant by the Fisher Exact Test (p=0.03) performed by Syracuse Research Corporation.

Several chronic animal studies reported no carcinogenic effects in rats, rabbits, or guinea pigs exposed to ≈1.6 mg chromium(VI)/m^3 as potassium dichromate or chromium dust 4 hours/day, 5 days/week (Baetjer et al. 1959b; Steffee and Baetjer 1965).

Rats exposed to ≤15.5 mg chromium(IV)/m^3 as chromium dioxide for 2 years had no statistically significant increased incidence of tumors (Lee et al. 1989).

The Cancer Effect Levels (CELs) are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 Death

Cases of accidental or intentional ingestion of chromium that have resulted in death have been reported in the past and continue to be reported even in more recent literature. In many cases, the amount of ingested chromium was unknown, but the case reports provide information on the sequelae leading to death. For example, a 22-month-old boy died 18.5 hours after ingesting an unknown amount of a sodium dichromate solution despite gastric lavage, continual attempts to resuscitate him from cardiopulmonary arrest, and other treatments at a hospital. Autopsy revealed generalized edema, pulmonary edema, severe bronchitis, acute bronchopneumonia, early hypoxic changes in the myocardium, liver congestion, and necrosis of the liver, renal tubules, and gastrointestinal tract (Ellis et al. 1982). Another case report of a 1-year-old girl who died after ingesting an unknown amount of ammonium dichromate reported severe dehydration, caustic burns in the mouth and pharynx, blood in the vomitus, diarrhea, irregular respiration, and labored breathing. The ultimate cause of death was shock and hemorrhage into the small intestine (Reichelderfer 1968).

Several reports were available in which the amount of ingested chromium (VI) compound could be estimated. A 17-year-old male died after ingesting 29 mg chromium(VI)/kg as potassium dichromate in a suicide. Despite attempts to save his life, he died 14 hours after ingestion from respiratory distress with severe hemorrhages. Caustic burns in the stomach and duodenum and gastrointestinal hemorrhage were also found (Clochesy 1984; Iserson et al. 1983). A 35-year-old female died after ingesting approximately 25 g chromium(VI) (357 mg chromium(VI)/kg assuming 70 kg body weight) as chromic acid in a suicide (Loubieres et al. 1999). The patient died of multiple organ failure. Terminal laboratory analysis and autopsy revealed metabolic acidosis, gastrointestinal hemorrhage and necrosis, fatty degeneration of the liver, and acute renal failure and necrosis.

A few reports have described death of humans after ingesting lower doses of chromium(VI). In one case, a 14-year-old boy died 8 days after admission to the hospital following ingestion of 7.5 mg chromium(VI)/kg as potassium dichromate from his chemistry set. Death was preceded by gastro-intestinal ulceration and severe liver and kidney damage (Kaufman et al. 1970). In another case, a

44-year-old man died of severe gastrointestinal hemorrhage 1 month after ingesting 4.1 mg chromium(VI)/kg as chromic acid (Saryan and Reedy 1988).

Acute oral LD_{50} values in rats exposed to chromium(III) or chromium(VI) compounds varied with the compound and the sex of the rat. LD_{50} values for chromium(VI) compounds (sodium chromate, sodium dichromate, potassium dichromate, and ammonium dichromate) range from 13 to 19 mg chromium(VI)/kg in female rats and from 21 to 28 mg chromium(VI)/kg in male rats (Gad et al. 1986). LD_{50} values of 108 (female rats) and 249 (male rats) mg chromium(VI)/kg for calcium chromate were reported by Vernot et al. (1977). The LD_{50} values for chromium trioxide were 25 and 29 mg chromium(VI)/kg for female and male rats, respectively (American Chrome and Chemicals 1989). An LD_{50} of 811 mg chromium(VI)/kg as strontium chromate was reported for male rats (Shubochkin and Pokhodzie 1980). Twenty percent mortality was observed when female Swiss Albino mice were exposed to potassium dichromate(VI) in drinking water at a dose of 169 mg chromium(VI)/kg/day (Junaid et al. 1996a). Similar exposure to a dose level of 89 mg chromium(VI)/kg/day resulted in 15% mortality among female rats of the Druckrey strain (Kanojia et al. 1998). The disparity between this dose and the LD_{50} identified in the Gad et al. (1986) study may be due to the route of administration, drinking water versus gavage. Chromium(III) compounds are less toxic than chromium(VI) compounds, with LD_{50} values in rats of 2,365 mg chromium(III)/kg as chromium acetate (Smyth et al. 1969) and 183 and 200 mg chromium(III)/kg as chromium nitrate in female and male rats, respectively (Vernot et al. 1977). The lower toxicity of chromium(III) acetate compared with chromium(III) nitrate may be related to solubility; chromium(III) acetate is less soluble in water than is chromium(III) nitrate. Signs of toxicity included hypoactivity, lacrimation, mydriasis, diarrhea, and change in body weight. Treatment with the chromium(III) dietary supplement chromium nicotinate of male and female rats resulted in no mortality at doses up to >621.6 mg/kg/day (Shara et al. 2005). The LD_{50} values for chromium(VI) or chromium(III) compounds indicate that female rats are slightly more sensitive to the toxic effects of chromium(VI) or chromium(III) than male rats. LD_{50} values in rats are recorded in Table 3-3 and plotted in Figure 3-3 for chromium(VI) and recorded in Table 3-4 and plotted in Figure 3-4 for chromium(III).

Intermediate and chronic exposure of rats and mice to chromium(III) or chromium(VI) compounds did not decrease survival. Survival was not affected in rats and mice exposed to chromium(VI) as sodium dichromate dihydrate in drinking water at doses up to 20.9 and 27.9 mg chromium(VI)/kg/day, respectively, for 3 months (NTP 2007) or at doses up to 7.0 and 8.7 mg chromium(VI)/kg/day,

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	once (IN)				29 M (death)	Clochesy 1984; Iserson et al. 1983 K2Cr2O7 (VI)	17-year-old, 60 kg boy ingested 5 g potassium dichromate [1,750 mg Cr(VI)]; dose = 29 mg Cr(VI)/kg.
2	Human	once (IN)				7.5 M (death)	Kaufman et al. 1970 K2Cr2O7 (VI)	14-year-old boy ingested 1.5 g potassium dichromate [0.53 mg Cr(VI)]. Assuming 70 kg body weight, dose = 7.5 mg Cr(VI)/kg.
3	Human	once (IN)				357 F (death)	Loubieres et al. 1999 CrO3 (VI)	35-year-old woman ingested chromic acid solution containing 25 g Cr(VI). Assuming 70 kg body weight, dose = 357 mg Cr(VI)/kg.
4	Human	once (IN)				4.1 M	Saryan and Reedy 1988 CrO3 (VI)	44-year-old man ingested ~2.8g Cr(VI) as chromium trioxide; ~4.1 mg Cr(VI)/kg body weight.
5	Rat (Fischer- 344)	once (G)				29 M (LD50) 25 F (LD50)	American Chrome and Chemicals 1989 CrO3 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
6	Rat (Fischer- 344)	once (GW)				21 M (LD50) 14 F (LD50)	Gad et al. 1986 Na2Cr2O7.2H2O (VI)	
7	Rat (Fischer- 344)	once (GW)				26 M (LD50) 17 F (LD50)	Gad et al. 1986 K2Cr2O7 (VI)	
8	Rat (Fischer- 344)	once (GW)				22 M (LD50) 19 F (LD50)	Gad et al. 1986 (NH4)2Cr2O7 (VI)	
9	Rat (Fischer- 344)	once (GW)				28 M (LD50) 13 F (LD50)	Gad et al. 1986 Na2CrO4 (VI)	
10	Rat Druckrey	2 wk (W)				89 F (15% mortality)	Kanojia et al. 1998 K2Cr2O7 (VI)	
11	Rat (NS)	once (G)				811 M (LD50)	Shubochkin and Pokhodzie 1980 SrCrO4 (VI)	
12	Rat (Sprague-Dawley)	once (G)				249 M (LD50) 108 F (LD50)	Vernot et al. 1977 CaCrO4 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
13	Human	once (IN)	Resp			29 M (congested lungs, pleural effusions)	Clochesy 1984; Iserson et al. 1983 K2Cr2O7 (VI)	17-year-old, 60 kg boy ingested 5 g potassium dichromate [1,750 mg Cr(VI)]; dose = 29 mg Cr(VI)/kg.
			Cardio			29 M (hemorrhage, cardiac arrest)		
			Gastro			29 M (hemorrhage)		
			Hemato			29 M (inhibited coagulation)		
			Renal			29 M (necrosis swelling of renal tubules)		
14	Human	once (IN)	Dermal		0.04 M (enhancement of dermatitis)		Goitre et al. 1982 K2Cr2O7 (VI)	
15	Human	once (C)	Dermal		0.036 (dermatitis)		Kaaber and Veien 1977 K2Cr2O7 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
16	Human	once (IN)	Gastro		7.5 M (abdominal pain and vomiting)		Kaufman et al. 1970 K2Cr2O7 (VI)	14-year-old boy ingested 1.5 g potassium dichromate [0.53 mg Cr(VI)]. Assuming 70 kg body weight, dose = 7.5 mg Cr(VI)/kg.
			Hepatic			7.5 M (necrosis)		
17	Human	once (IN)	Gastro			357 F (intestinal hemorrhage and necrosis)	Loubieres et al. 1999 CrO3 (VI)	35-year-old woman ingested chromic acid solution containing 25 g Cr(VI). Assuming 70 kg body weight, dose = 357 mg Cr(VI)/kg.
			Hepatic			357 F (fatty degeneration)		
			Renal			357 F (acute renal failure and renal necrosis)		
			Metab			357 F (metabolic acidosis)		
18	Human	once (IN)	Gastro			4.1 M (gastrointestinal hemorrhage)	Saryan and Reedy 1988 CrO3 (VI)	44-year-old man ingested ~2.8g Cr(VI) as chromium trioxide; ~4.1 mg Cr(VI)/kg body weight.
			Renal			4.1 M (acute tubular necrosis)		

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
19	Rat (Fischer- 344)	5 d (W)	Hemato		4 M (decreased mean cell volume, mean cell hemoglobin, and reticulocyte count)		NTP 2007 Na2Cr2O7.2H2O (VI)	
			Musc/skel	15.9 M 8.2 F	31.8 M (serum creatine kinase activity increased by 31%)			
					16.4 F (serum creatine kinase activity increased by 45%)			
			Hepatic		4 M (serum ALT activity increased by 15%)			
20	Rat (Fischer- 344)	4 d (W)	Hemato	0.7 M	2.8 M (decreased mean cell hemoglobin)		NTP 2008a Na2Cr2O7.2H2O (VI)	
			Renal	19.3 M				
21	Rat (NS)	once (G)	Gastro			130 (hemorrhage)	Samitz 1970 K2Cr2O7 (VI)	
22	Mouse (Swiss albino)	9 d Gd 6-14 (W)	Bd Wt	53.2 F	101.1 F (8.2% decrease in gestational weight gain)	152.4 F (24.3% decrease in gestational weight gain)	Junaid et al. 1996b K2Cr2O7 (VI)	
Immuno/ Lymphoret								
23	Human	once (IN)			0.04 M (enhancement of chromium dermatitis)		Goitre et al. 1982 K2Cr2O7 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
24	Human	once (C)			0.036 (dermatitis)		Kaaber and Veien 1977 K2Cr2O7 (VI)	
Neurological								
25	Human	once (IN)				7.5 M (cerebral edema)	Kaufman et al. 1970 K2Cr2O7 (VI)	14-year-old boy ingested 1.5 g potassium dichromate [0.53 mg Cr(VI)]. Assuming 70 kg body weight, dose = 7.5 mg Cr(VI)/kg.
Reproductive								
26	Rat (NS)	3 d Gd 1-3 (G)				35.7 F (preimplanation loss)	Bataineh et al. 2007 K2Cr2O7 (VI)	
27	Rat (NS)	3 d Gd 4-6 (G)				35.7 F (decreased number of viable fetuses; increased resorptions)	Bataineh et al. 2007 K2Cr2O7 (VI)	
28	Rat (Wistar)	6 d (G)				5.2 M (sperm count decreased by 76%, percentage of abnormal sperm increased by 143% and histopathological changes to seminiferous tubules)	Li et al. 2001 CrO3 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
29	Rat (Wistar)	Gd 6-15 (W)				8 F (increased pre- and post-implantation loss, resorptions, dead fetuses/litter, skeletal and visceral malformations)	Elsaieed and Nada 2002 K2CrO4 (VI)	
30	Mouse (Swiss albino)	9 d Gd 6-14 (W)				53.2 F (increase in resorptions)	Junaid et al. 1996b K2Cr2O7 (VI)	
INTERMEDIATE EXPOSURE								
Death								
31	Mouse (Swiss albino)	20 d (W)				169 F (3/15 died)	Junaid et al. 1996a K2Cr2O7 (VI)	
Systemic								
32	Rat (Wistar)	22 wk (W)	Hepatic			1.3 M (increased serum ALT and AST and histopathological changes, including degeneration, vacuolization, increased sinusoidal space and necrosis)	Acharya et al. 2001 K2Cr2O2 (VI)	
			Renal		1.3 M (histopathological changes, including vacuolization in glomeruli, degeneration of basement membrane of Bowman's capsule and renal tubular epithelial degeneration)			

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
33	Rat (Sprague-Dawley)	12 wk (W)	Bd Wt		42 M (19% lower final body weight)		Bataineh et al. 1997 K2Cr2O7 (VI)	
34	Rat Charles Foster	90 d 1 x/d (G)	Bd Wt	20 M		40 M (57% decreased body weight)	Chowdhury and Mitra 1995 Na2Cr2O7 (VI)	
35	Rat (Wistar)	28 d (W)	Renal	10 M		100 M (proteinuria, oliguria)	Diaz-Mayans et al. 1986 Na2CrO4 (VI)	
36	Rat Swiss albino	20 d (W)	Bd Wt	37	70 (14% reduced maternal body weight gain)	87 (21% reduced maternal body weight gain)	Kanojia et al. 1996 K2Cr2O7 (VI)	
37	Rat Druckrey	3 mo (W)	Bd Wt	45	89 (18% reduced maternal body weight gain)	124 (24% reduced maternal body weight gain)	Kanojia et al. 1998 K2Cr2O7 (VI)	
38	Rat (albino)	20 d 7 d/wk (G)	Hepatic		13.5 M (lipid accumulation)		Kumar and Rana 1982 K2CrO4 (VI)	
			Renal		13.5 M (lipid accumulation)			

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
39	Rat (white)	20 d 7 d/wk (G)	Renal		13.5 M (inhibition of membrane enzymes; alkaline phosphatase, acid phosphatase, lipase)		Kumar and Rana 1984 K2CrO4 (VI)	
40	Rat (albino)	20 d 7 d/wk (G)	Hepatic		13.5 M (changes in liver enzyme activities; inhibition of acid phosphatase; enhancement of lipase)		Kumar et al. 1985 K2CrO4 (VI)	
41	Rat (Sprague-Dawley)	9 wk (F)	Hemato	2.1 M	8.4 M		NTP 1996b K2Cr2O7 (VI)	
				2.5 F	9.8 F (decreased mean corpuscular volume)			
			Hepatic	9.8				
			Renal	9.8				
			Bd Wt	9.8				

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
42	Rat (Fischer- 344)	14 wk (W)	Resp	20.9				NTP 2007 Na2Cr2O7.2H2O (VI)	
			Cardio	20.9					
			Gastro	1.7	3.5	(duodenal histiocytic cellular infiltration)			
			Hemato		1.7	(microcytic, hypochromic anemia)			
			Musc/skel	3.5	5.9	(serum creatine kinase activity increased by 31% in males and 45% in females)			
			Hepatic		1.7	(serum ALT activity increased by 14% in males 30% in females, serum SDH activity increased by 77% in males and 359% in females)			
			Renal	20.9					
			Endocr	20.9					
			Ocular	20.9					
			Bd Wt	5.9 M	11.2 M	(11% decrease in body weight)			

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
43	Rat (Fischer- 344)	23 d (W)	Hemato		1.7 M (decreased hematocrit, mean cell volume, mean hemoglobin concentration, reticulocyte)		NTP 2007 Na2Cr2O7.2H2O (VI)	
					1.7 F (decreased hemoglibin and mean cell volume)			
44	Rat (Fischer- 344)	6 mo (W)	Hemato	0.21 M	0.77 M (microcytic, hypochromic anemia)		NTP 2008a Na2Cr2O7.2H2O (VI)	
45	Rat (Fischer- 344)	22 d (W)	Hemato	0.21 M	0.77 M[b] (microcytic, hypochromic anemia)		NTP 2008a Na2Cr2O7.2H2O (VI)	
46	Rat (Wistar)	30 d (W)	Endocr		73 M (59% decrease in serum prolactin)		Quinteros et al. 2007 K2Cr2O7 (VI)	
			Bd Wt		73 M (11.6% in body wieght)			

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
47	Rat (Wistar)	10 wk (W)	Hepatic			3.7 M (serum ALT activity increased by 253%, histopathological changes including focal necrosis and degeneration with changes in vascularization)	Rafael et al. 2007 Cr (VI)	
			Metab		3.7 M (65% increase in serum glucose)			
48	Mouse BDF1	210 d (W)	Bd Wt	1.4 F	14 F (13.5% decrease in body weight gain)		De Flora et al. 2006 Na2Cr2O7.2H2O (VI)	
49	Mouse (BALB/c)	9 wk (F)	Hemato	7.4 M 12 F	32.2 M 48 F (decreased mean corpuscular volume)		NTP 1996a K2Cr2O7 (VI)	
			Hepatic	1.1 M 1.8 F	3.5 M 5.6 F (cytoplasmic vacuolization of hepatocytes)			
			Renal	48				
			Bd Wt	48				

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
50	Mouse (BALB/c)	85 d + pnd 1-74 (F1) + pnd 1-21(F2) (F)	Gastro	36.7 F			NTP 1997 K2Cr2O7 (VI)	
			Hemato		7.8 F (decreased mean corpuscular volume in F1)			
			Hepatic	36.7 F				
			Renal	36.7 F				
			Bd Wt	36.7 F				

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
51	Mouse (B6C3F1)	14 wk (W)	Resp	27.9			NTP 2007 Na2Cr2O7.2H2O (VI)	
			Cardio	27.9				
			Gastro		3.1 (epithelial hyperplasia of duodenum)			
			Hemato		3.1 M (decreased mean cell volume)			
					3.1 F (decreased mean cell hemoglobin)			
			Hepatic	27.9				
			Renal	27.9				
			Endocr	27.9				
			Ocular	27.9				
			Bd Wt	3.1 F	3.1 M (6% decrease in body weight)			
					5.2 F (8% decrease in body weight)			
52	Mouse (B6C3F1)	22 d (W)	Hemato		0.38 F (microcytic, hypochromic anemia and increased lymphocytes)		NTP 2008a Na2Cr2O7.2H2O (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
53	Mouse (B6C3F1)	6 mo (W)	Hemato	0.38 F	1.4 F (decreased mean cell volume)		NTP 2008a Na2Cr2O7.2H2O (VI)	
54	Mouse (albino)	19 d (W)	Bd Wt	46 F	98 F (decreased maternal weight gain)		Trivedi et al. 1989 K2Cr2O7 (VI)	
55	Rabbit (New Zealand)	daily 10 wk (G)	Bd Wt	3.6 M			Yousef et al. 2006 K2Cr2O7 (VI)	
Immuno/ Lymphoret								
56	Rat (Fischer- 344)	14 wk (W)		11.2 F	1.7 M (histiocytic cellular infilratration of pancreatic lymph nodes)		NTP 2007 Na2Cr2O7.2H2O (VI)	
					20.9 F (histiocytic cellular infiltration of pancreatic lymph nodes)			
57	Rat (Fischer- 344)	3-10 wk (W)			16 (increased proliferation of T- and B- lymphocytes in response to mitogens and antigens)		Snyder and Valle 1991 K2CrO4 (VI)	
58	Mouse (B6C3F1)	14 wk (W)			3.1 (histiocytic infiltrate of mesenteric lymph nodes)		NTP 2007 Na2Cr2O7.2H2O (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
59	Rat (Wistar)	28 d (W)		10 M	100 M (decreased motor activity)		Diaz-Mayans et al. 1986 Na2CrO4 (VI)	
60	Rat (Fischer- 344)	14 wk (W)		20.9			NTP 2007 Na2Cr2O7.2H2O (VI)	
61	Mouse (B6C3F1)	14 wk (W)		27.9			NTP 2007 Na2Cr2O7.2H2O (VI)	
Reproductive								
62	Monkey macaca	180 d (W)				2.1 M (histopathological changes to epididymides, including ductal obstruction and development of microcanals)	Aruldhas et al. 2004 K2Cr2O7 (VI)	
63	Monkey macaca	180 d (W)				2.1 M (decreased testes weight, histopathological changes including depletion of germ cells, hyperplasia of Leydig cells, disrupted spermatogenesis, Sertoli cell fibrosis, alterations of sperm morpholologgy)	Aruldhas et al. 2005 K2Cr2O7 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
64	Monkey macaca	180 d (W)				2.1 M (histopathological changes to basal cells and principal cells of epididymis)	Aruldhas et al. 2006 K2Cr2O7 (VI)	
65	Monkey macaca	180 d (W)		1.1 M		2.1 M (sperm count and motility decreased by 25%)	Subramanian et al. 2006 K2Cr2O7 (VI)	
66	Rat (Sprague-Dawley)	12 wk (W)			42 (altered sexual behavior, decreased absolute testes, seminal vesicles, and preputial gland weights)		Bataineh et al. 1997 K2Cr2O7 (VI)	
67	Rat (Charles Foster)	90 d 1 x/d (G)			20 M (decreased testicular protein, 3 beta-hydroxy steroid dehydrogenase and serum testosterone)	40 M (28% decreased testicular weight; decreased testicular protein, DNA, RNA, seminiferous tubular diameter; decreased Leydig cells, pachytene cells, spermatocytes, spermatids, and testosterone levels)	Chowdhury and Mitra 1995 Na2Cr2O7 (VI)	
68	Rat Swiss albino	20 d (W)				37 (increased resorptions)	Kanojia et al. 1996 K2Cr2O7 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
69	Rat Druckrey	3 mo (W)				45 (decreased fertility, increased pre- and post-implantation loss)	Kanojia et al. 1998 K2Cr2O7 (VI)	
70	Rat (Sprague-Dawley)	9 wk (F)		8.4 M 9.8 F			NTP 1996b K2Cr2O7 (VI)	
71	Rat (Fischer- 344)	14 wk (W)		20.9			NTP 2007 Na2Cr2O7.2H2O (VI)	
72	Mouse (Swiss albino)	20 d (W)			52 F (decreased placental weight)	98 F (preimplantation loss, increased resorptions)	Junaid et al. 1996a K2Cr2O7 (VI)	
73	Mouse Swiss albino	20 d (W)			60 F (decreased number of follicles at different stages of maturation)	120 F (decreased number of ova/mouse)	Murthy et al. 1996 K2Cr2O7 (VI)	
74	Mouse (BALB/c)	9 wk (F)		32.2 M 48 F			NTP 1996a K2Cr2O7 (VI)	
75	Mouse (BALB/c)	85 d + pnd 1-74 (F1) + pnd 1-21(F2) (F)		36.7 F			NTP 1997 K2Cr2O7 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
76	Mouse (B6C3F1)	14 wk (W)		27.9			NTP 2007 Na2Cr2O7.2H2O (VI)	
77	Mouse (B6C3F1)	14 wk (W)		8.7 M			NTP 2007 Na2Cr2O7.2H2O (VI)	
78	Mouse (albino)	Gd 1-19 19 d (W)				46 F (increase in fetal resorption and post implantation loss)	Trivedi et al. 1989 K2Cr2O7 (VI)	
79	Mouse (BALB/c)	7 wk 7 d/wk (F)				15.2 M (decreased spermatogenesis)	Zahid et al. 1990 K2Cr2O7 (VI)	
80	Rabbit (New Zealand)	daily 10 wk (G)				2.6 M (plasma testosterone decreased by 20.8%, sperm count decreased by 18%, % dead sperm increased by 23.9%, total mobile sperm decreased by 34.3%)	Yousef et al. 2006 K2Cr2O7 (VI)	
Developmental								
81	Rat Swiss albino	20 d (W)				37 (increased post-implantation loss and decreased number of live fetuses)	Kanojia et al. 1996 K2Cr2O7 (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
82	Rat Druckrey	3 mo (W)				45 (reduced fetal caudal ossification, increased post-implantation loss, reduced fetal weight, subhemorrhagic patches)	Kanojia et al. 1998 K2Cr2O7 (VI)	
83	Mouse (BALB/c)	Gd 12- Ld 20 (W)			66 F (delayed time of vaginal opening and impaired fertility in female offspring)		Al-Hamood et al. 1998 K2Cr2O7 (VI)	
84	Mouse BDF1	Gd 0-18 (W)		4.8 F			De Flora et al. 2006 Na2Cr2O7.2H2O (VI)	
85	Mouse BDF1	Gd 0-18 (W)		2.4 F			De Flora et al. 2006 K2Cr2O7 (VI)	
86	Mouse (Swiss albino)	20 d (W)				52 F (reduced caudal ossification in fetuses; decreased fetal weight; post-implantation loss)	Junaid et al. 1996a K2Cr2O7 (VI)	
87	Mouse (albino)	Gd 1-19 19 d (W)				46 (increased resorptions, reduced ossification, gross anomalies)	Trivedi et al. 1989 K2Cr2O7 (VI)	
CHRONIC EXPOSURE								
Death								
88	Rat (Fischer- 344)	2 yr (W)		7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
89	Mouse (B6C3F1)	2 yr (W)		8.7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	
Systemic								
90	Human	NS (environ)	Gastro		0.57 (oral ulcer, diarrhea, abdominal pain, indigestion, vomiting)		Zhang and Li 1987 (VI)	Exposed to well water containing 20 mg Cr(VI)/L; assuming 70 kg body weight and drinking water consumption of 2 L/day, dose = 0.57 mg Cr(VI)/kg/day.
			Hemato		0.57 (leukocytosis, immature neutrophils)			
91	Rat (Sprague-Dawley)	1 yr (W)	Hemato	3.6			MacKenzie et al. 1958 K2CrO4 (VI)	
			Hepatic	3.6				
			Renal	3.6				
			Bd Wt	3.6				
92	Rat (Fischer- 344)	12 mo (W)	Hemato	0.21 M	0.77 M (decreased mean cell hemoglobin)		NTP 2008a Na2Cr2O7.2H2O (VI)	
			Musc/skel	0.94 M	2.4 M (creatine kinase activity increased by 64%)			
			Hepatic	0.21 M	0.77 M (serum ALT increased by 156%)			

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
93	Rat (Fischer- 344)	2 yr (W)	Resp	7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	
			Cardio	7 F				
			Gastro	0.21 M 0.94 F	0.77 M (histiocytic cellular infiltration of duodenum) 2.4 F (histiocytic cellular infiltrate of duodenum)			
			Hepatic	0.21 M	0.77 M (basophilic foci of liver) 0.24 F (chronic inflammation)			
			Renal	7 F				
			Endocr	7 F				
			Ocular	7 F				
			Bd Wt	2.1 M	5.9 M (12% decrease in body weight)			
94	Mouse (B6C3F1)	1 yr (W)	Hemato	1.4 F	3.1 F (increased RBC count, decreased mean cell volume and mean cell hemoglobin)		NTP 2008a Na2Cr2O7.2H2O (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
95	Mouse (B6C3F1)	2 yr (W)	Resp	8.7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	
			Cardio	8.7 F				
			Gastro		0.38[c] (epithelial hyperplasia of duodenum in males and female and cytoplasmic alteration of pancreas in females)			
			Hepatic	2.4 M	5.9 M (clear cell and eosinophilic foci)			
					0.38 F (histiocytic cellular infiltration)			
			Renal	8.7 F				
			Endocr	8.7 F				
			Ocular	8.7 F				
Immuno/ Lymphoret								
96	Rat (Fischer- 344)	2 yr (W)		0.21 M 0.94 F	0.77 M (histiocytic cellular infiltration and hemorrhage of mesenteric nodes)		NTP 2008a Na2Cr2O7.2H2O (VI)	
					2.4 F (histiocytic cellular infiltration of mesenteric and pancreatic nodes)			

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
97	Mouse (B6C3F1)	2 yr (W)			0.38 (histiocytic cellular infiltration of mesenteric lymph nodes)		NTP 2008a Na2Cr2O7.2H2O (VI)	
Neurological								
98	Rat (Fischer- 344)	2 yr (W)		7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	
99	Mouse (B6C3F1)	2 yr (W)		8.7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	
Reproductive								
100	Rat (Fischer- 344)	2 yr (W)		6.6 M 7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	
101	Mouse (B6C3F1)	2 yr (W)		5.9 M 8.7 F			NTP 2008a Na2Cr2O7.2H2O (VI)	

Table 3-3 Levels of Significant Exposure to Chromium VI - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Cancer								
102	Human	(environ)				0.57 (CEL: lung and stomach cancer)	Zhang and Li 1987 Cr (VI)	Exposed to well water containing 20 mg Cr(VI)/L; assuming 70 kg body weight and drinking water consumption of 2 L/day, dose = 0.57 mg Cr(VI)/kg/day.
103	Rat (Fischer- 344)	2 yr (W)				5.9 M (CEL: neoplasm of squamous epithelium of mouth and tongue) 7 F (CEL: neoplasm of squamous cell epithelium of mouth and tongue)	NTP 2008a Na2Cr2O7.2H2O (VI)	
104	Mouse (B6C3F1)	2 yr (W)				3.1 M (CEL: neoplastic lesions of small intestine) 2.4 M (CEL: neoplastic lesions of small intestine)	NTP 2008a Na2Cr2O7.2H2O (VI)	

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

b Used to derive an intermediate-duration oral MRL of 0.005 mg chromium(VI)/kg/day for chromium(VI) compounds. The benchmark dose of 0.52 mg/kg/day (average of the benchmark doses derived for MCV, MCH, and Hgb) was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

c Used to derive a chronic-duration oral MRL of 0.001 mg chromium(VI)/kg/day for chromium(VI) compounds. Benchmark dose of 0.09 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

(VI) = hexavalent; avg = average; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; environ = environmental; (F) = feed; F = female; F1 = first generation; F2 = second generation; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; (IN) = ingestion; Ld = lactational day; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; (occup) = occupational; pnd = post natal day; Resp = respiratory; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; (W) = drinking water; wk = week(s); x = times; yr = year(s)

Figure 3-3 Levels of Significant Exposure to Chromium VI - Oral

Acute (≤14 days)

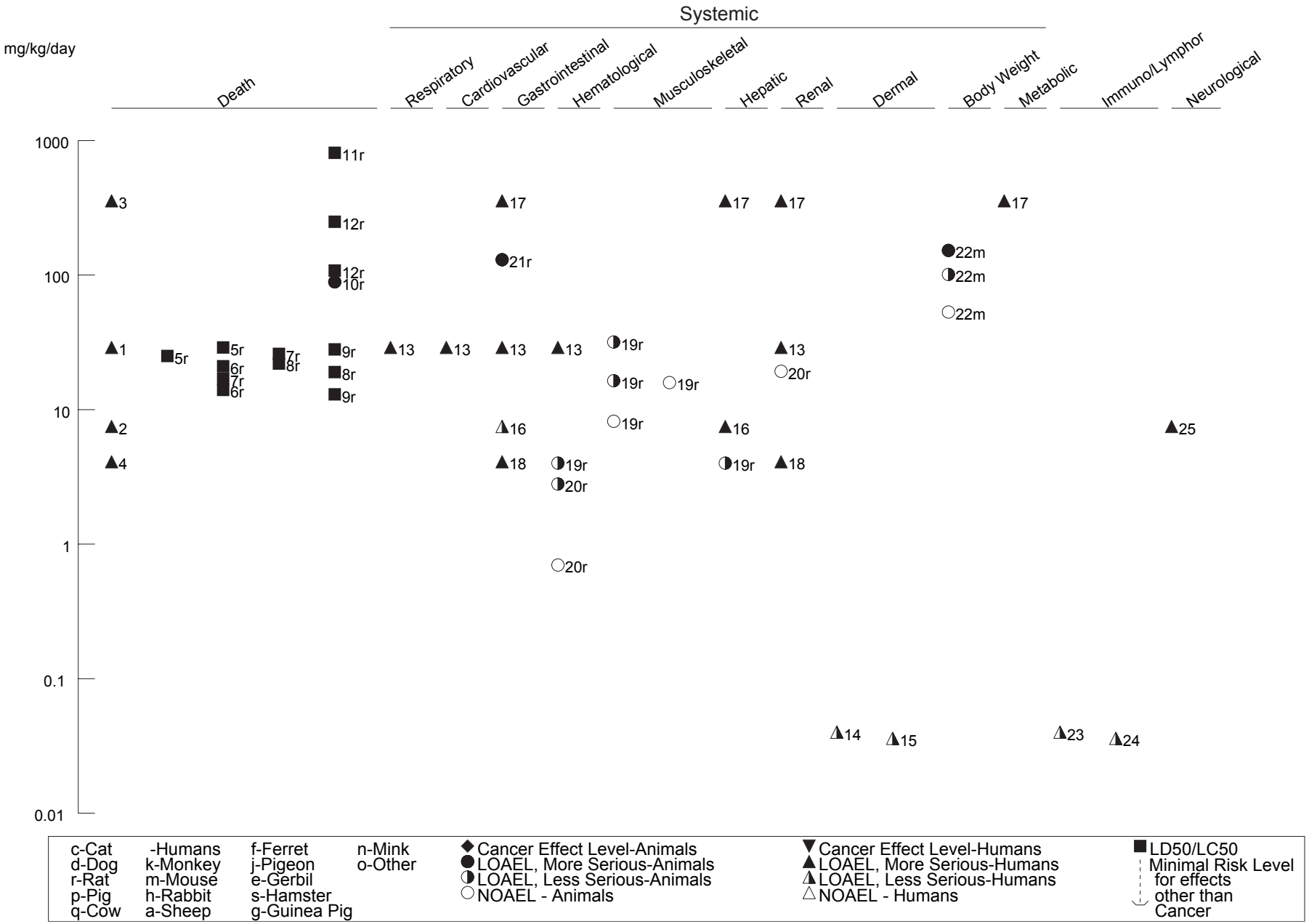


Figure 3-3 Levels of Significant Exposure to Chromium VI - Oral *(Continued)*

Acute (≤14 days)

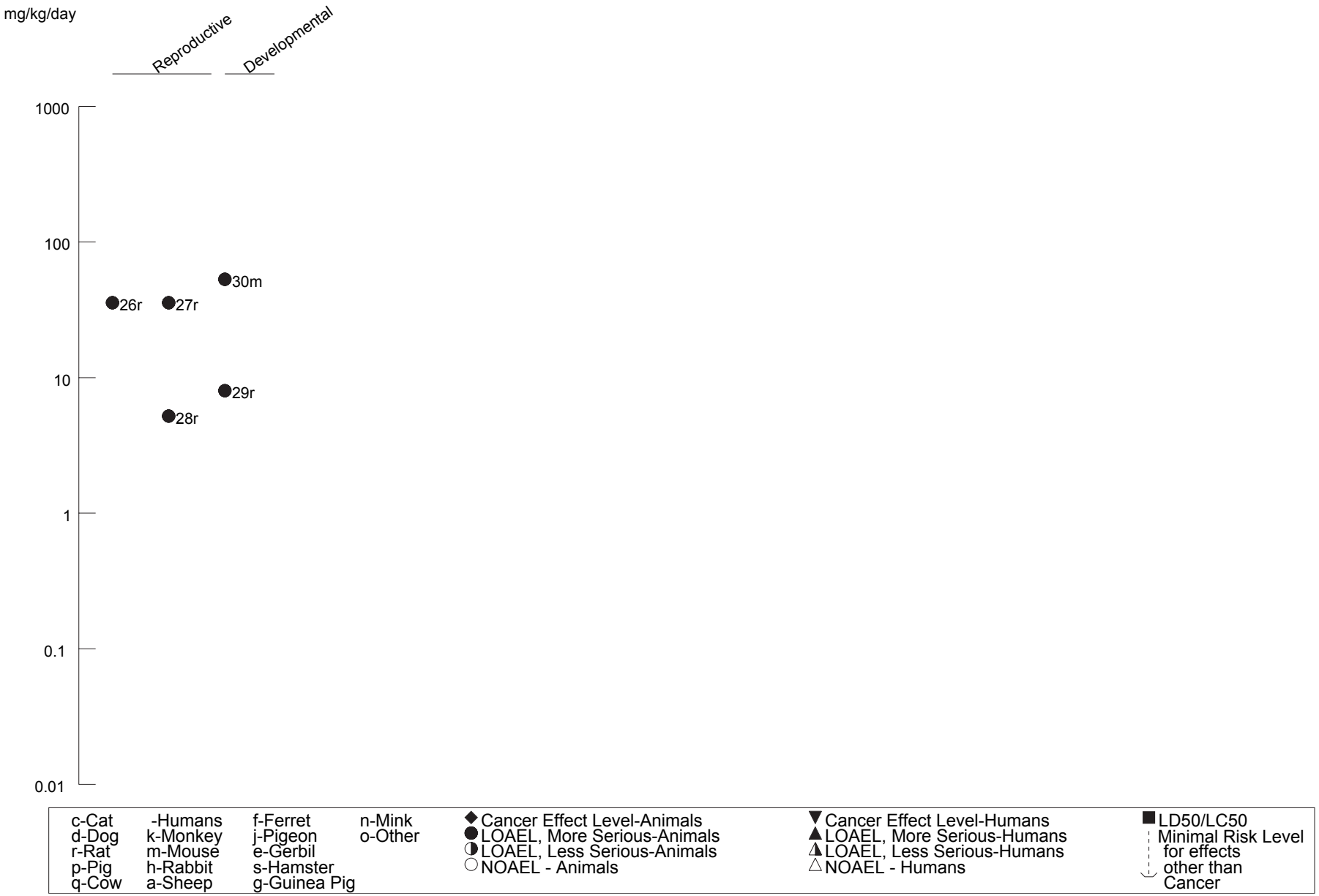


Figure 3-3 Levels of Significant Exposure to Chromium VI - Oral *(Continued)*

Intermediate (15-364 days)

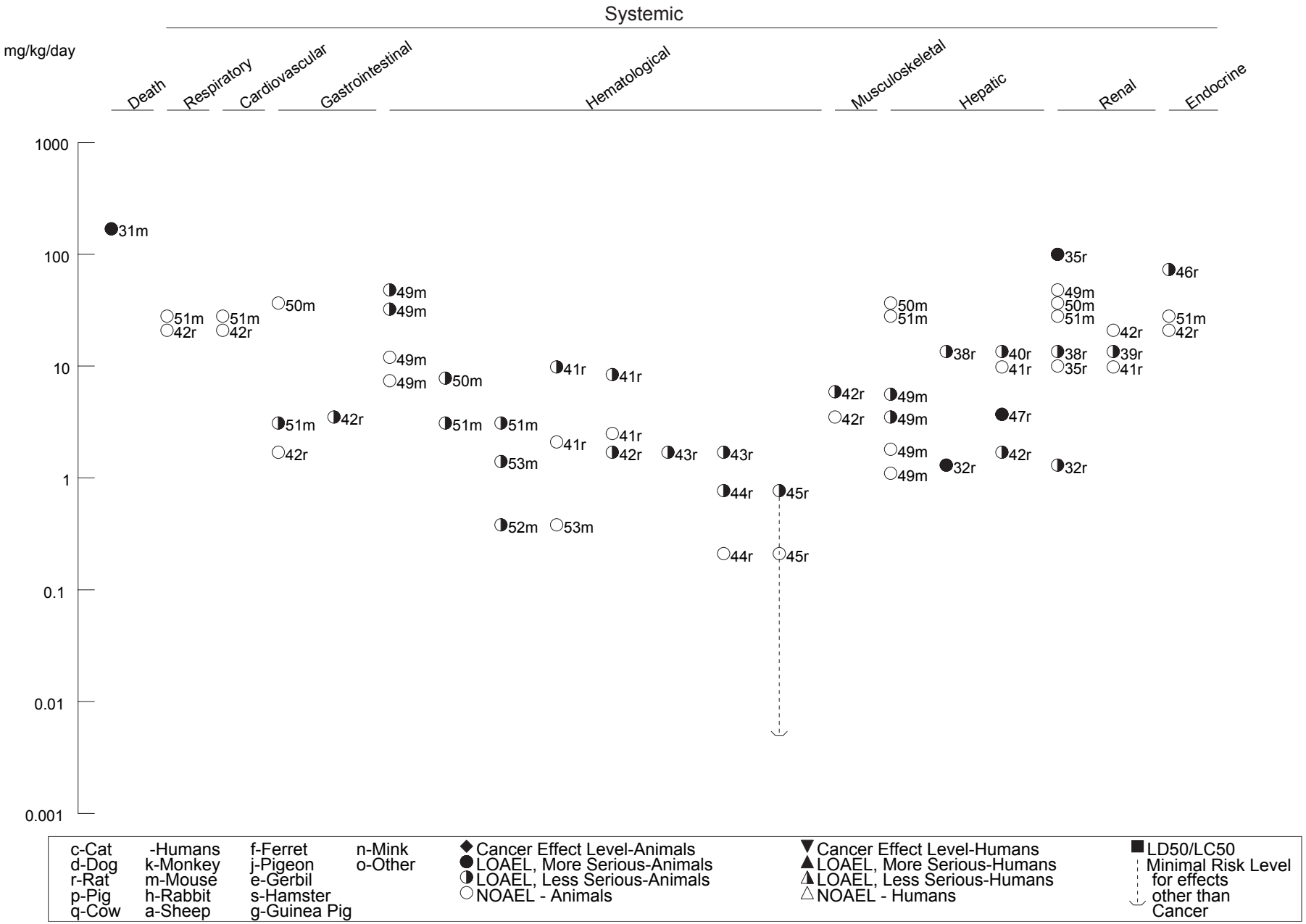


Figure 3-3 Levels of Significant Exposure to Chromium VI - Oral *(Continued)*

Intermediate (15-364 days)

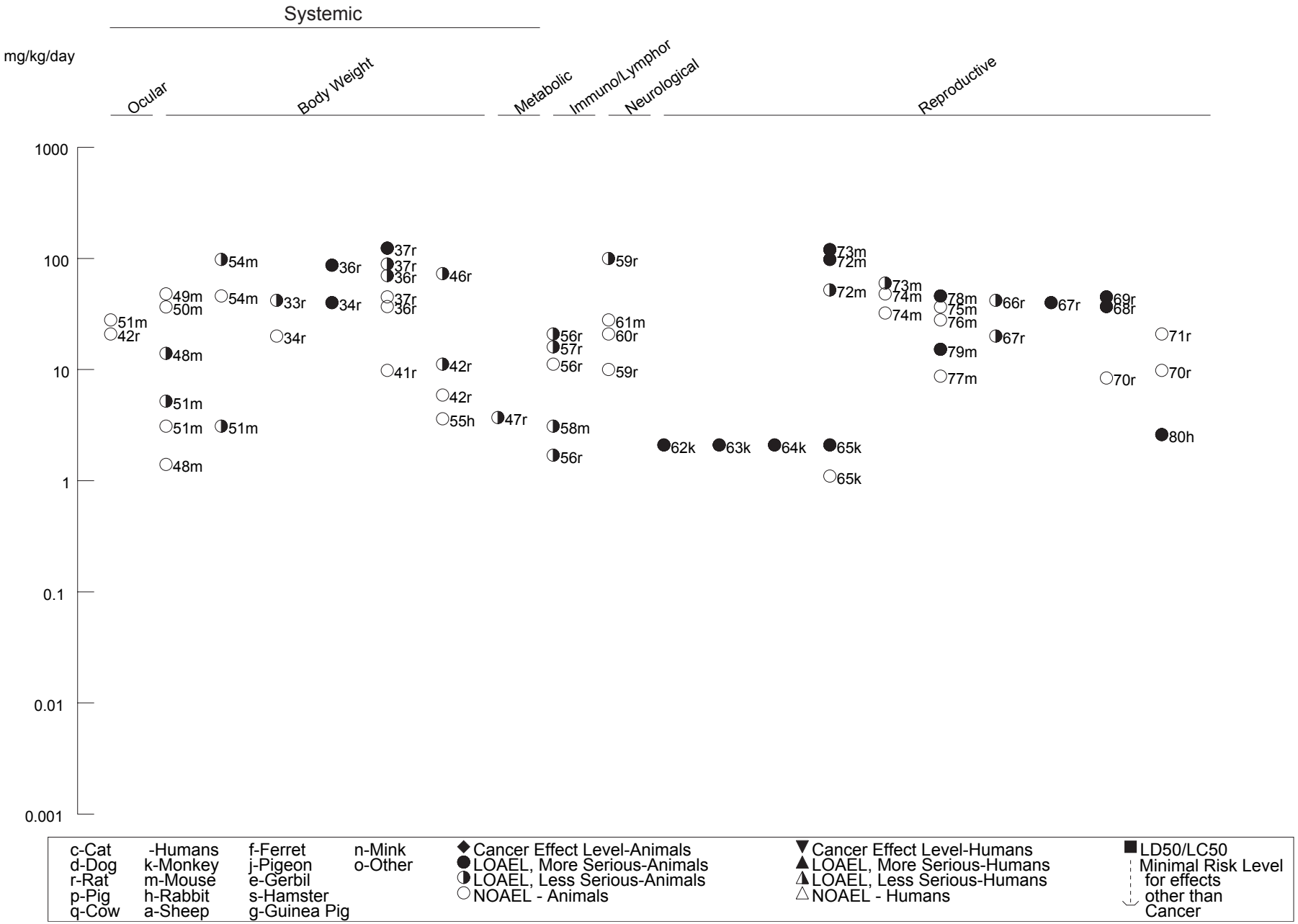


Figure 3-3 Levels of Significant Exposure to Chromium VI - Oral *(Continued)*

Intermediate (15-364 days)

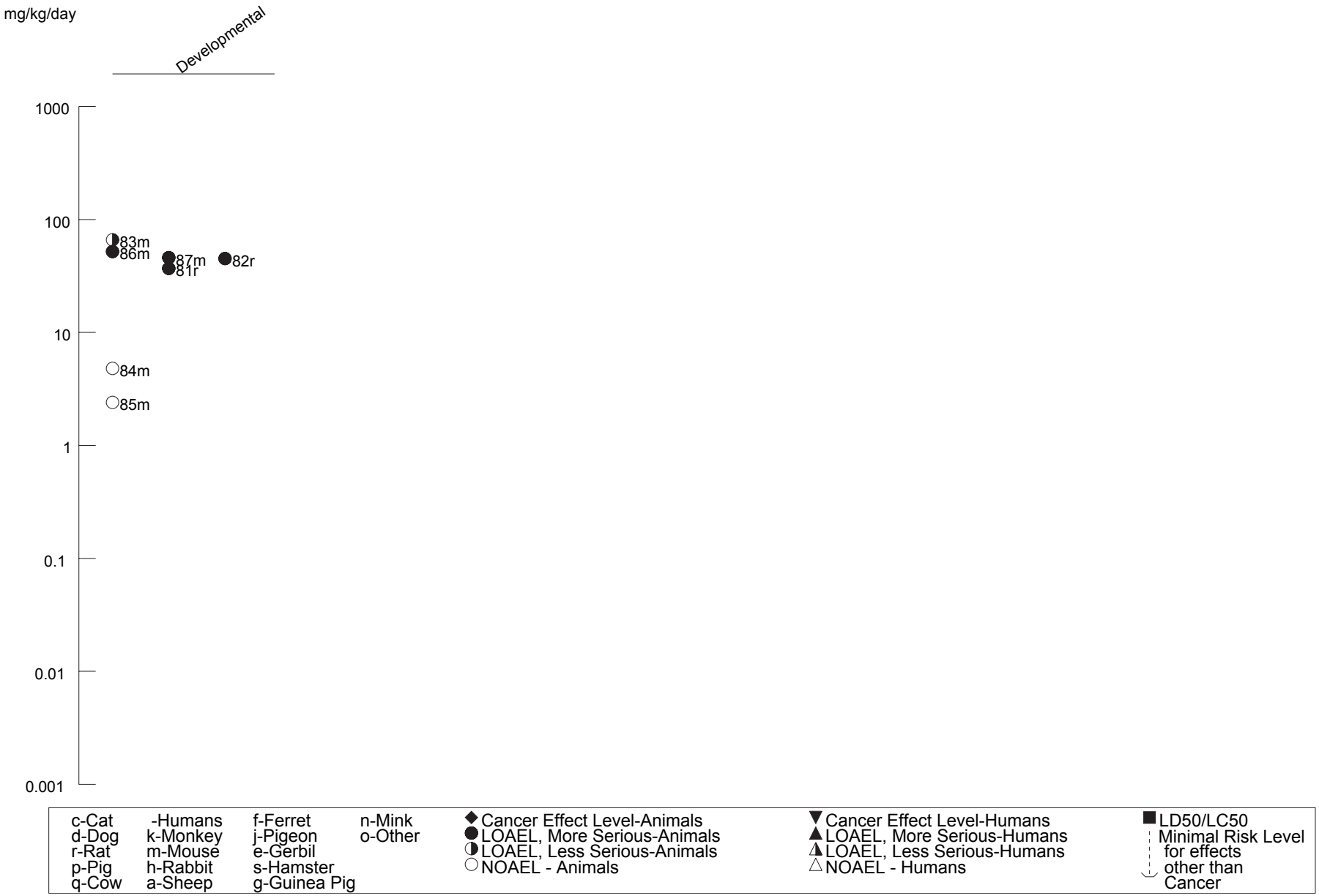


Figure 3-3 Levels of Significant Exposure to Chromium VI - Oral *(Continued)*

Chronic (≥365 days)

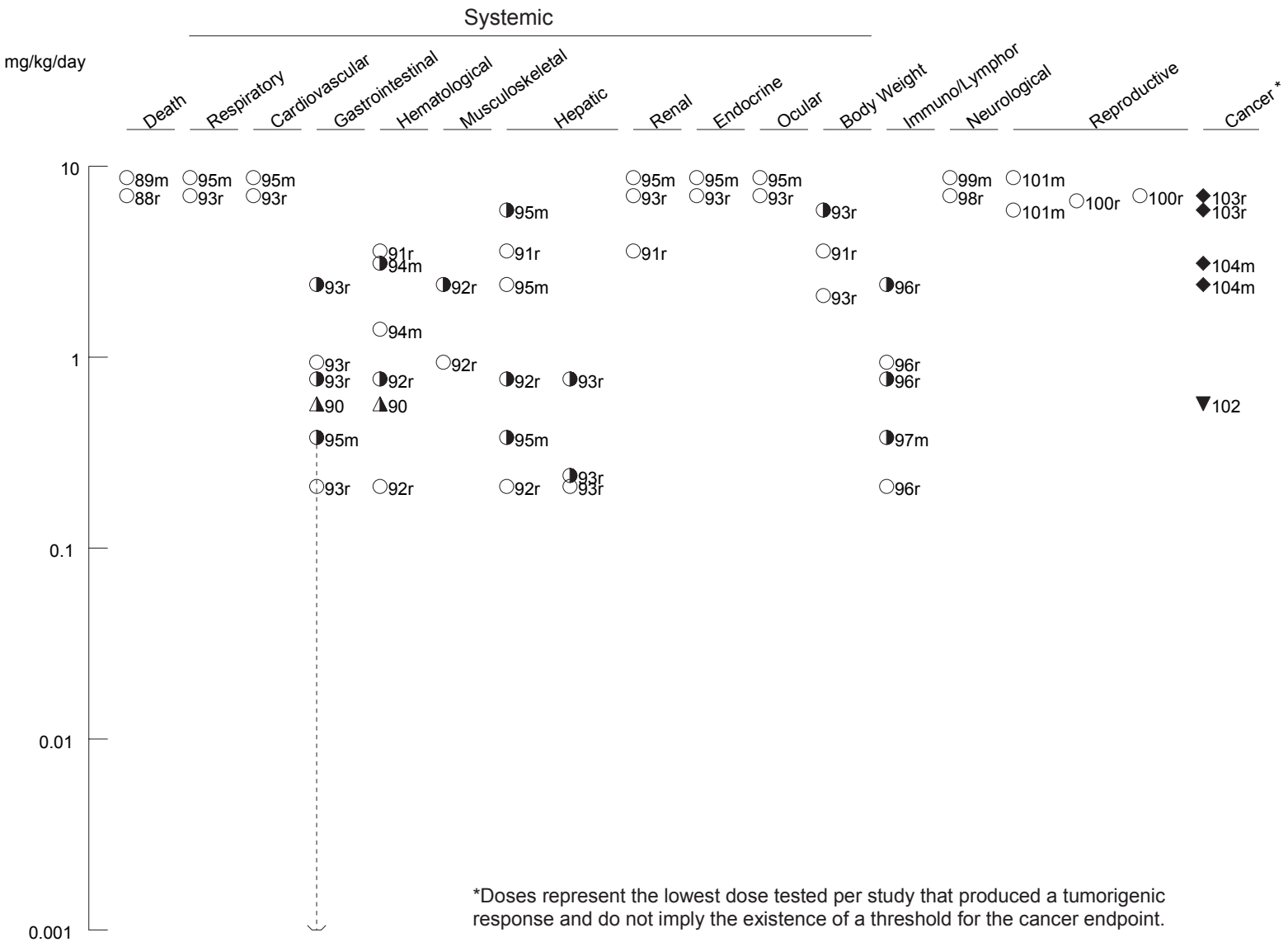


Table 3-4 Levels of Significant Exposure to Chromium III - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (NS)	once (GW)				2365 (LD50)	Smyth et al. 1969 Cr(CH3COO)3H2O (III)	
2	Rat (Sprague-Dawley)	once (G)				200 M (LD50) 183 F (LD50)	Vernot et al. 1977 Cr(NO3)3.9H2O (III)	
Systemic								
3	Rat (Fischer- 344)	3 d (F)	Hemato	506 F			NTP 2008 Cr picolinate (III)	
Reproductive								
4	Rat (NS)	3 d Gd 1-3 (G)				33.6 F (decreased number of pregnancies)	Bataineh et al. 2007 CrCl3 (III)	
5	Rat (NS)	3 d Gd 4-6 (G)		33.6 F			Bataineh et al. 2007 CrCl3 (III)	
INTERMEDIATE EXPOSURE								
Systemic								
6	Rat (Sprague-Dawley)	daily 20 wk (F)	Hepatic	9			Anderson et al. 1997b CrCl3 (III)	
			Renal	9				
			Bd Wt	9				

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Rat (Sprague-Dawley)	daily 20 wk (F)	Hepatic	9			Anderson et al. 1997b Cr picolinate (III)	
			Renal	9				
			Bd Wt	9				
8	Rat (Sprague-Dawley)	12 wk (W)	Bd Wt			40 (24% lower final body weight)	Bataineh et al. 1997 CrCl3 (III)	
9	Rat (BD)	90 d 5 d/wk (F)	Resp	1806			Ivankovic and Preussmann 1975 Cr2O3 (III)	
			Cardio	1806				
			Gastro	1806				
			Hemato	1806				
			Hepatic	1806				
			Renal	1806				

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
10	Rat (Fischer- 344)	14 wk (F)	Resp	506 F			NTP 2008 Cr picolinate (III)	
			Cardio	506 F				
			Gastro	506 F				
			Hemato	506 F				
			Hepatic	506 F				
			Renal	506 F				
			Endocr	506 F				
			Ocular	506 F				
			Bd Wt	506 F				
11	Rat (Sprague-Dawley)	90 d (F)	Resp	1.5 F			Shara et al. 2005 Cr nicotinate (III)	
			Cardio	1.5 F				
			Gastro	1.5 F				
			Hemato	1.5 F				
			Hepatic	1.5 F				
			Renal	1.5 F				
			Endocr	1.5 F				
			Bd Wt	1.5 F				

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
12	Rat (Sprague-Dawley)	38 wk (F)	Resp	0.25 F			Shara et al. 2007 Cr nicotinate (III)	
			Cardio	0.25 F				
			Gastro	0.25 F				
			Hemato	0.25 F				
			Hepatic	0.25 F				
			Renal	0.25 F				
			Endocr	0.25 F				
13	Mouse BDF1	210 d (W)	Bd Wt	165 M 140 F			De Flora et al. 2006 CrK(SO4)2 (III)	
14	Mouse (Swiss)	12 wk (W)	Bd Wt	14 F	5 M (14% decrease in body weight gain)		Elbetieha and Al-Hamood 1997 CrCl3 (III)	

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Mouse (B6C3F1)	14 wk (F)	Resp	1415 M			NTP 2008 Cr picolinate (III)	
			Cardio	1415 M				
			Gastro	1415 M				
			Hemato	1415 M				
			Hepatic	1415 M				
			Renal	1415 M				
			Endocr	1415 M				
			Ocular	1415 M				
			Bd Wt	1415 M				
Immuno/ Lymphoret								
16	Rat (Fischer- 344)	14 wk (F)		506 F			NTP 2008 Cr picolinate (III)	
17	Rat (Sprague-Dawley)	90 d (F)		1.5 F			Shara et al. 2005 Cr nicotinate (III)	
18	Rat (Sprague-Dawley)	38 wk (F)		0.25 F			Shara et al. 2007 Cr nicotinate (III)	
19	Mouse (B6C3F1)	14 wk (F)		1415 M			NTP 2008 Cr picolinate (III)	
Neurological								
20	Rat	90 d 5 d/wk (F)		1806			Ivankovic and Preussmann 1975 Cr III	

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
21	Rat (Fischer- 344)	14 wk (F)		506 F			NTP 2008 Cr picolinate (III)	
22	Rat (Fischer- 344)	14 wk ad lib (F)		506 F			NTP 2008 Cr picolinate (III)	
23	Rat (Sprague-Dawley)	90 d (F)		1.5 F			Shara et al. 2005 Cr nicotinate (III)	
24	Rat (Sprague-Dawley)	38 wk (F)		0.25 F			Shara et al. 2007 Cr nicotinate (III)	
25	Mouse (B6C3F1)	14 wk ad lib (F)		1415 M			NTP 2008 Cr picolinate (III)	
Reproductive								
26	Rat (Sprague-Dawley)	12 wk (W)			40 (altered sexual behavior, decreased absolute testes, seminal vesicles, and preputial gland weights)		Bataineh et al. 1997 CrCl3 (III)	
27	Rat (Fischer- 344)	14 wk (F)		506 F			NTP 2008 Cr picolinate (III)	
28	Rat (Sprague-Dawley)	90 d (F)		1.5 F			Shara et al. 2005 Cr nicotinate (III)	

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
29	Rat (Sprague-Dawley)	38 wk (F)		0.25 F			Shara et al. 2007 Cr nicotinate (III)	
30	Mouse (Swiss)	12 wk (W)			5 M (increased testes and decreased preputial gland weights)	5 F (decreased number of implantations and viable fetuses; increased ovarian and decreased uterine weights)	Elbetieha and Al-Hamood 1997 CrCl3 (III)	
31	Mouse (B6C3F1)	14 wk (F)		1415 M			NTP 2008 Cr picolinate (III)	
32	Mouse (BALB/c)	7 wk 7 d/wk (F)				9.1 M (decreased spermatogenesis)	Zahid et al. 1990 Cr2(SO4)3 (III)	
Developmental								
33	Rat (BD)	90 d 5 d/wk (F)		1806			Ivankovic and Preussmann 1975 Cr2O3 (III)	
34	Mouse (BALB/c)	Gd 12- Ld 20 (W)			74 (reduced ovary and testis weights in offspring and impaired fertility in female offspring)		Al-Hamood et al. 1998 CrCl3 (III)	

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Systemic								
35	Rat (BD)	2 yr 5 d/wk (F)	Resp	2040			Ivankovic and Preussmann 1975 Cr2O3 (III)	
			Cardio	2040				
			Gastro	2040				
			Hepatic	2040				
			Renal	2040				
36	Rat (Sprague-Dawley)	1 yr (W)	Hemato	3.6			MacKenzie et al. 1958 CrCl3 (III)	
			Hepatic	3.6				
			Renal	3.6				
			Bd Wt	3.6				
37	Rat (Fischer- 344)	2 yr (F)	Resp	313 F			NTP 2008 Cr picolinate (III)	
			Cardio	313 F				
			Gastro	313 F				
			Hepatic	313 F				
			Renal	313 F				
			Endocr	313 F				
			Ocular	313 F				
			Bd Wt	313 F				

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
38	Rat (Long- Evans)	2-3 yr 7 d/wk (W)	Cardio	0.46			Schroeder et al. 1965 Cr(CH3COO)3 (III)	
			Hepatic	0.46				
			Renal	0.46				
			Bd Wt	0.46				
39	Rat (Sprague-Dawley)	52 wk (F)	Resp	0.25 F			Shara et al. 2007 Cr nicotinate (III)	
			Cardio	0.25 F				
			Gastro	0.25 F				
			Hemato	0.25 F				
			Hepatic	0.25 F				
			Renal	0.25 F				
			Endocr	0.25 F				
			Bd Wt		0.22 M (14.9% decrease in body weight)			
					0.25 F (9.6% decrease in body weight)			

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
40	Mouse (B6C3F1)	2 yr (F)	Resp	781 M			NTP 2008 Cr picolinate (III)	
			Cardio	781 M				
			Gastro	781 M				
			Hepatic	781 M				
			Renal	781 M				
			Endocr	781 M				
			Ocular	781 M				
			Bd Wt	781 M				
Immuno/ Lymphoret								
41	Rat (Fischer- 344)	2 yr (F)		313 F			NTP 2008 Cr picolinate (III)	
42	Rat (Sprague-Dawley)	52 wk (F)		0.25 F			Shara et al. 2007 Cr nicotinate (III)	
43	Mouse (B6C3F1)	2 yr (F)		781 M			NTP 2008 Cr picolinate (III)	
Neurological								
44	Rat	2 yr 5 d/wk (F)		2040			Ivankovic and Preussmann 1975 Cr III	
45	Rat (Fischer- 344)	2 yr (F)		313 F			NTP 2008 Cr picolinate (III)	

Table 3-4 Levels of Significant Exposure to Chromium III - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
46	Rat (Sprague-Dawley)	52 wk (F)		0.25 F			Shara et al. 2007 Cr nicotinate (III)	
47	Mouse (B6C3F1)	2 yr (F)		781 M			NTP 2008 Cr picolinate (III)	
Reproductive								
48	Rat (Fischer- 344)	2 yr (F)		313 F			NTP 2008 Cr picolinate (III)	
49	Rat (Sprague-Dawley)	52 wk (F)		0.25 F			Shara et al. 2007 Cr nicotinate (III)	
50	Mouse (B6C3F1)	2 yr (F)		781 M			NTP 2008 Cr picolinate (III)	
Cancer								
51	Rat (Fischer- 344)	2 yr (F)				55 M (equivocal evidence for prepubital gland adenoma)	NTP 2008 Cr picolinate (III)	

a The number corresponds to entries in Figure 3-4.

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gn Pig = guinea pig; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; ppd = post-parturition day; ppm = parts per million; Resp = respiratory; x = time(s); (W) = drinking water; wk = week(s); yr = year(s)

Figure 3-4 Levels of Significant Exposure to Chromium III - Oral

Acute (≤14 days)

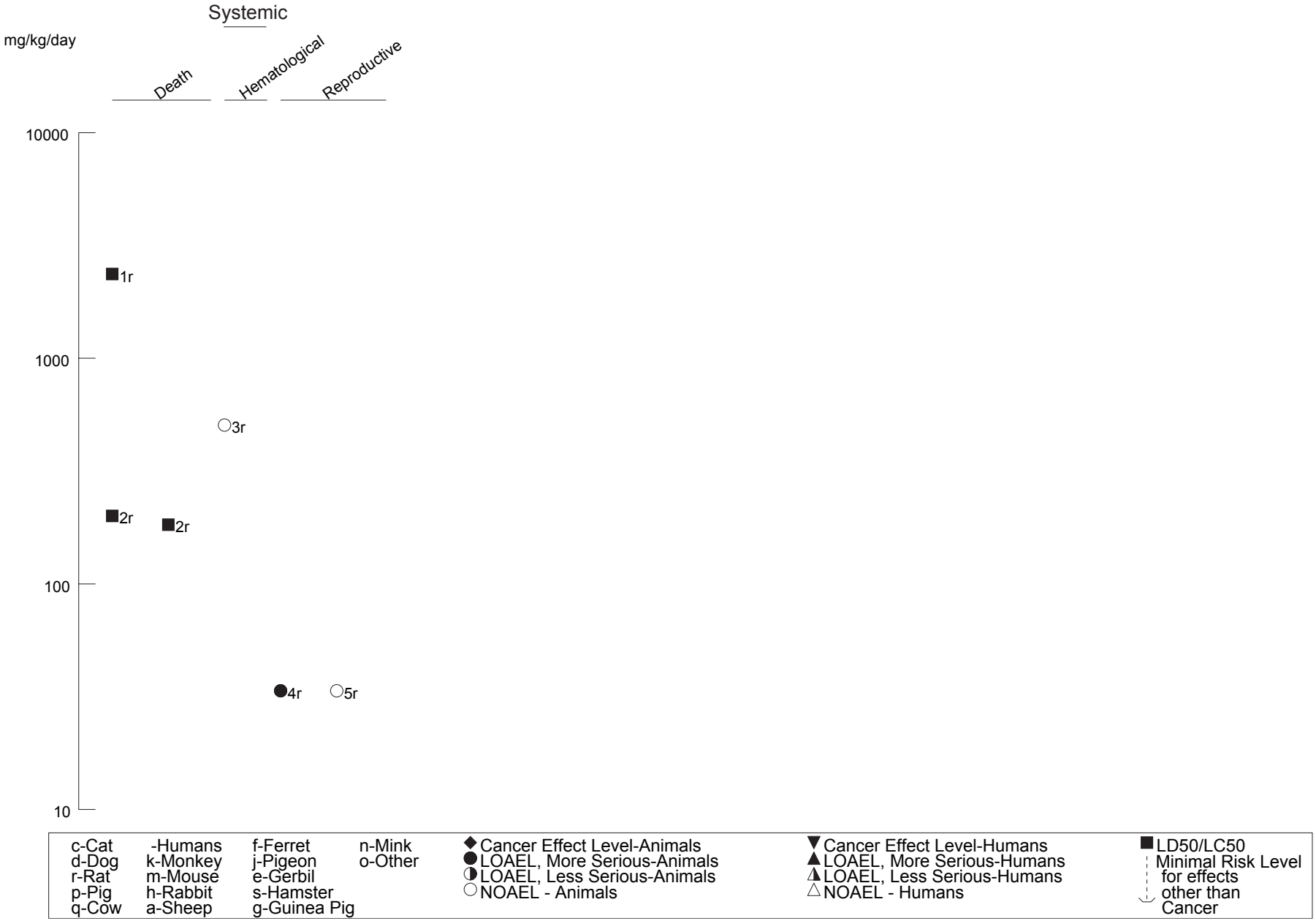


Figure 3-4 Levels of Significant Exposure to Chromium III - Oral *(Continued)*

Intermediate (15-364 days)

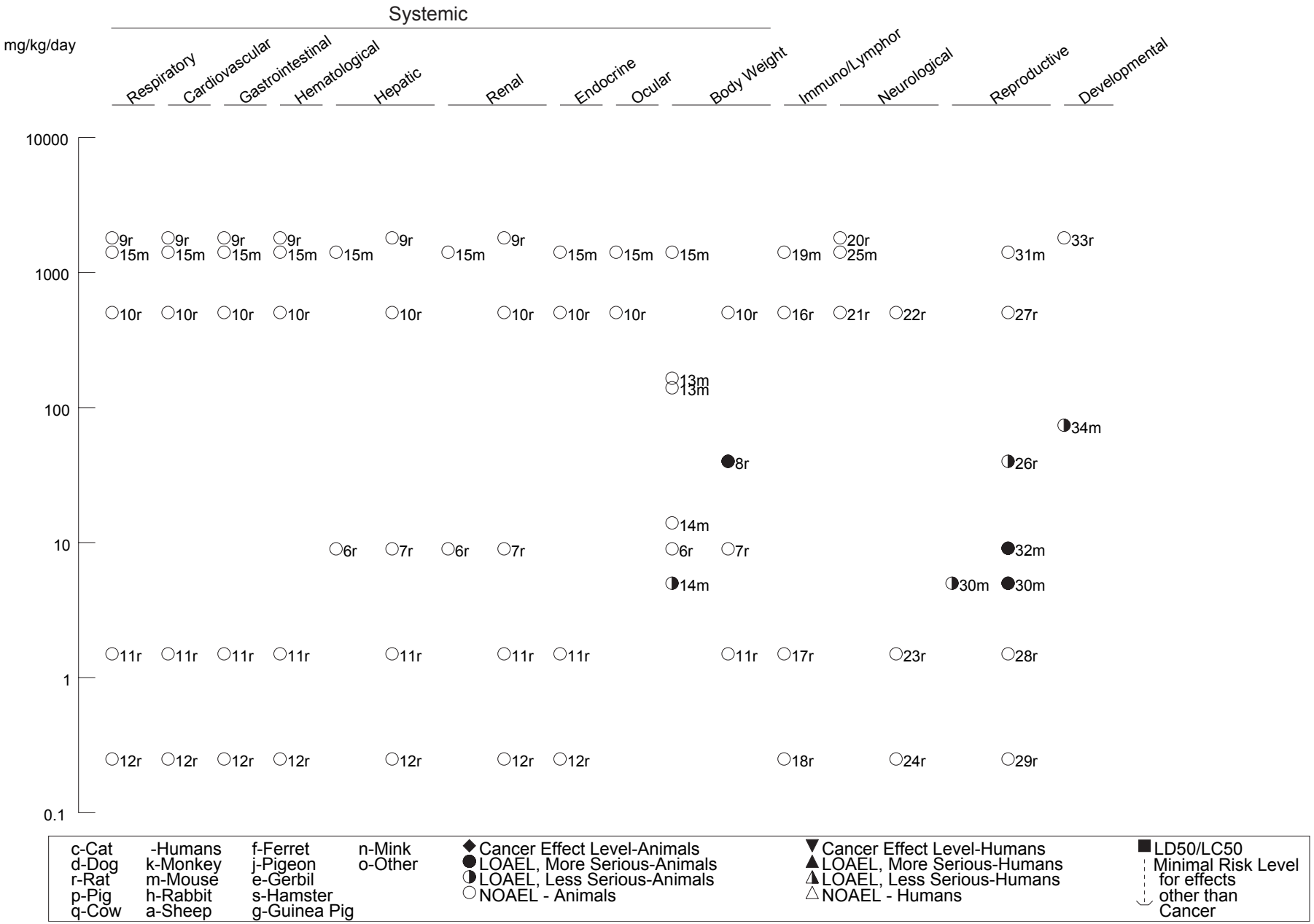
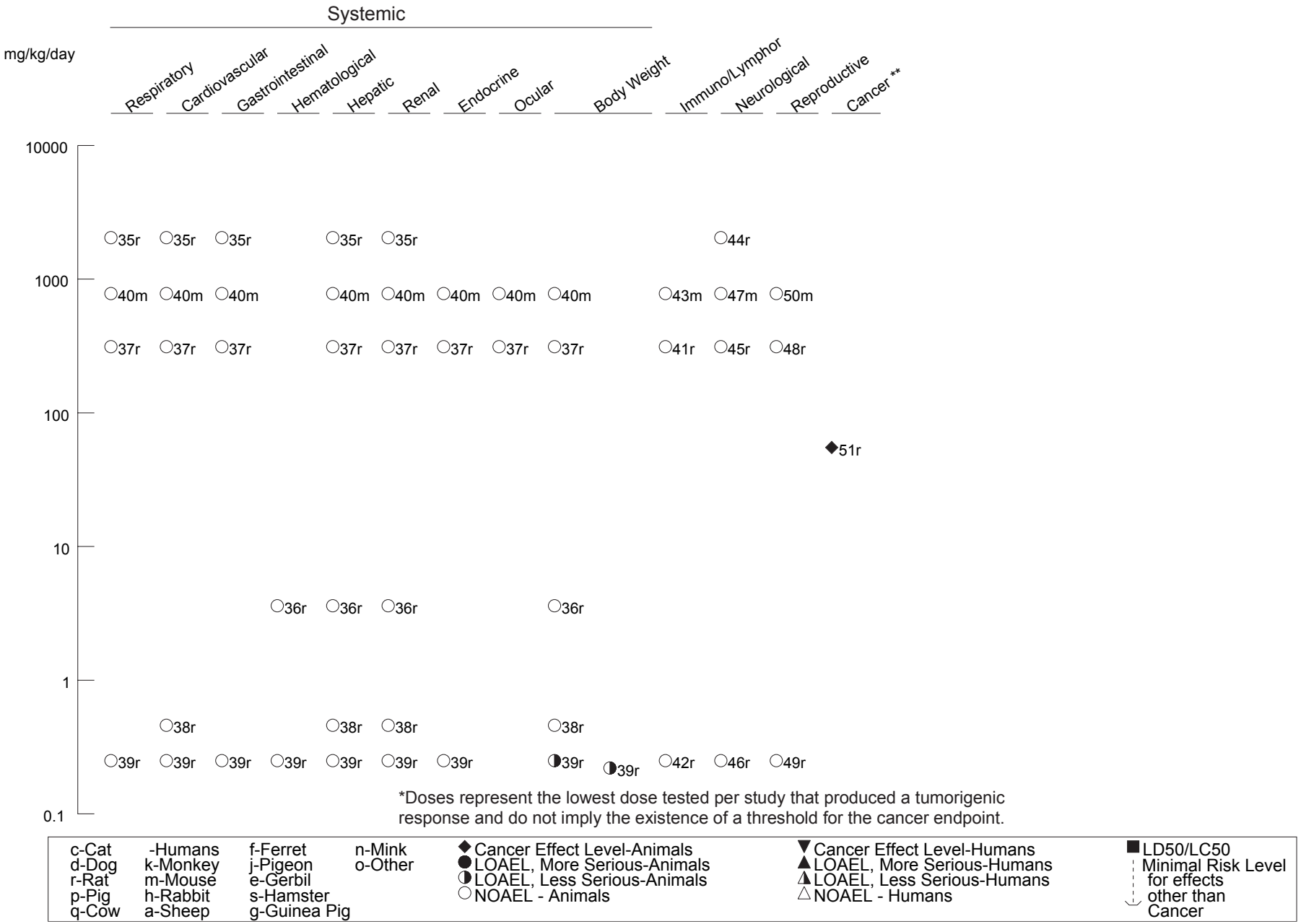


Figure 3-4 Levels of Significant Exposure to Chromium III - Oral *(Continued)*

Chronic (≥365 days)



respectively, for 2 years (NTP 2008a). Mortality was not increased in rats fed 2,040 mg chromium(III)/kg/day as chromium oxide in the diet 5 days/week for 2 years (Ivankovic and Preussmann 1975) or in rats and mice fed up to 313 and 781 mg chromium(III)/kg/day, respectively, as chromium picolinate in the diet for 2 years (NTP 2008b).

3.2.2.2 Systemic Effects

The systemic effects of oral exposure to chromium(III) and chromium(VI) compounds are discussed below. The highest NOAEL values and all reliable LOAEL values for each systemic effect in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3 for chromium(VI) and recorded in Table 3-4 and plotted in Figure 3-4 for chromium(III).

Respiratory Effects. Case reports of humans who died after ingesting chromium(VI) compounds have described respiratory effects as part of the sequelae leading to death. A 22-month-old boy who ingested an unknown amount of sodium dichromate died of cardiopulmonary arrest. Autopsy revealed pleural effusion, pulmonary edema, severe bronchitis, and acute bronchopneumonia (Ellis et al. 1982). Autopsy of a 17-year-old male who committed suicide by ingesting 29 mg chromium(VI)/kg as potassium dichromate revealed congested lungs with blood-tinged bilateral pleural effusions (Clochesy 1984; Iserson et al. 1983). Respiratory effects were not reported at nonlethal doses. No information was identified on respiratory effects in humans after oral exposure to chromium(III) compounds.

No studies were identified regarding respiratory function in animals after oral exposure to chromium(VI) or chromium(III) compounds. The histopathology of lung and nasal tissue has been evaluated in rats and mice exposed to oral chromium(VI) (as sodium dichromate dihydrate) and chromium(III) (as chromium nicotinate, chromium oxide and chromium picolinate) for durations of 3 months to 2 years, with no abnormalities observed (Ivankovic and Preussmann 1975; NTP 2007, 2008a, 2008b; Shara et al. 2005, 2007). For chromium(VI) compounds, the highest doses tested for intermediate and chronic exposure durations were 27.9 and 8.7 chromium(VI)/kg/day, respectively, as sodium dichromate dihydrate in drinking water (NTP 2007, 2008a). For chromium(III) compounds, the highest doses tested for intermediate and chronic exposure durations were 1,806 and 2,040 mg chromium(III)/kg/day, respectively, as chromium oxide in the diet 5 days/week (Ivankovic and Preussmann 1975).

Cardiovascular Effects. Case reports of humans who died after ingesting chromium(VI) compounds have described cardiovascular effects as part of the sequelae leading to death. A 22-month-old boy who

ingested an unknown amount of sodium dichromate died of cardiopulmonary arrest. Autopsy revealed early hypoxic changes in the myocardium (Ellis et al. 1982). In another case, cardiac output, heart rate, and blood pressure dropped progressively during treatment in the hospital of a 17-year-old male who had ingested 29 mg chromium(VI)/kg as potassium dichromate. He died of cardiac arrest. Autopsy revealed hemorrhages in the anterior papillary muscle of the left ventricle (Clochesy 1984; Iserson et al. 1983). Cardiovascular effects have not been reported at nonlethal doses. No information was identified on cardiovascular effects in humans after oral exposure to chromium(III) compounds.

No studies were located regarding effects on cardiovascular function in animals after oral exposure to chromium(VI) compounds. Histopathological examination of the heart has been evaluated in rats and mice exposed to oral chromium(VI) (as sodium dichromate dihydrate and sodium acetate) and chromium(III) (as chromium nicotinate, chromium oxide and chromium picolinate) for durations of 3 months to 2 years, with no abnormalities observed (Ivankovic and Preussmann 1975; NTP 2007, 2008a, 2008b; Schroeder et al. 1965; Shara et al. 2005, 2007). For chromium(VI) compounds, the highest doses tested for intermediate and chronic exposure durations were 27.9 and 8.7 chromium(VI)/kg/day, respectively, as sodium dichromate dihydrate in drinking water (NTP 2007, 2008a). For chromium(III) compounds, the highest doses tested for intermediate and chronic exposure durations were 1,806 and 2,040 mg chromium(III)/kg/day, respectively, as chromium oxide in the diet 5 days/week (Ivankovic and Preussmann 1975). None of these studies assessed cardiovascular end points such as blood pressure or electrocardiograms.

Gastrointestinal Effects. Cases of gastrointestinal effects in humans after oral exposure to chromium(VI) compounds have been reported. In one study, a 14-year-old boy who died after ingesting 7.5 mg chromium(VI)/kg as potassium dichromate experienced abdominal pain and vomiting before death. Autopsy revealed gastrointestinal ulceration (Kaufman et al. 1970). In another study, a 44-year-old man died of gastrointestinal hemorrhage after ingesting 4.1 mg chromium(VI)/kg as chromic acid solution (Saryan and Reedy 1988). Gastrointestinal hemorrhage and extensive necrosis of all digestive mucous membranes were also observed on autopsy of a 35-year-old woman who died following ingestion of 357 mg chromium(VI)/kg as chromic acid (Loubieres et al. 1999). Gastrointestinal burns and hemorrhage have also been described as contributing to the cause of death of infants who ingested unknown amounts of sodium dichromate (Ellis et al. 1982) or ammonium dichromate (Reichelderfer 1968) and a 17-year-old male who ingested ~29 mg chromium(VI)/kg as potassium dichromate (Clochesy 1984; Iserson et al. 1983).

Some chromium(VI) compounds, such as potassium dichromate and chromium trioxide, are caustic and irritating to mucosal tissue. A 25-year-old woman who drank a solution containing potassium dichromate experienced abdominal pain and vomited (Goldman and Karotkin 1935). Two people who ate oatmeal contaminated with potassium dichromate became suddenly ill with severe abdominal pain and vomiting, followed by diarrhea (Partington 1950). Acute gastritis developed in a chrome plating worker who had accidentally swallowed an unreported volume of a plating fluid containing 300 g chromium trioxide/L. He was treated by hemodialysis, which saved his life (Fristedt et al. 1965). Nausea, hemetemesis, and bloody diarrhea were reported in a 24-year-old woman who ingested ammonium dichromate in a suicide attempt (Hasan 2007).

Ingestion of chromium compounds as a result of exposure at the workplace has occasionally produced gastrointestinal effects. In a chrome plating plant where poor exhaust resulted in excessively high concentrations of chromium trioxide fumes, in addition to symptoms of labored breathing, dizziness, headache, and weakness from breathing the fumes during work, workers experienced nausea and vomiting upon eating on the premises (Lieberman 1941). Gastrointestinal effects were also reported in an epidemiology study of 97 workers in a chromate plant exposed to dust containing both chromium(III) and chromium(VI) compounds. Blocked nasal passages, as a result of working in the dust laden atmosphere, forced the individuals to breathe through their mouths, thereby probably ingesting some of the chromium dust. A 10.3% incidence of gastric ulcer formation and a 6.1% incidence of hypertrophic gastritis was reported. Epigastric and substernal pain were also reported in the chromate production workers (Mancuso 1951). Gastric mucosa irritation resulting in duodenal ulcer, possibly as a result of mouth breathing, has also been reported in other studies of chromate production workers (Sassi 1956; Sterekhova et al. 1978). Subjective symptoms of stomach pain, duodenal ulcers, gastritis, stomach cramps, and indigestion were reported by workers exposed to a mean concentration of 0.004 mg chromium(VI)/m^3 in an electroplating facility where zinc, cadmium, nickel, tin, and chromium plating were carried out (Lucas and Kramkowski 1975). An otolaryngological examination of 77 employees of eight chromium electroplating facilities in Czechoslovakia, where the mean level in the breathing zone above the plating baths was 0.414 mg chromium(VI)/m^3, revealed 12 cases of chronic tonsillitis, 5 cases of chronic pharyngitis, and 32 cases of atrophic changes in the left larynx (Hanslian et al. 1967). These effects were probably also due to exposure via mouth breathing.

In a cross-sectional study conducted in 1965 of 155 villagers whose well water contained chromium(VI)/L as a result of pollution from an alloy plant in the People's Republic of China, associations were found between drinking the contaminated water and oral ulcer, diarrhea, abdominal

pain, indigestion, and vomiting. The alloy plant began chromium smelting in 1961 and began regular production in 1965. Similar results were found in two similar studies in other villages, but further details were not provided (Zhang and Li 1987). The highest concentration of chromium(VI) detected during sampling was 20 mg chromium(VI)/L, equivalent to a dose of 0.57 mg chromium(VI)/kg/day based on a default reference water consumption rate and body weight value of 2 L/day and 70 kg, respectively (note that these values may not be appropriate for the Chinese study population). However, exposure estimates for this population are uncertain and it is likely that exposure levels in many cases were to concentrations less than 20 mg chromium(VI)/L. At least some residents obtained drinking water from alternative sources (Sedman et al. 2006) and exposure may have been self-limiting due to lack of palatability of water (Beaumont et al. 2008). Thus, exposure levels associated with adverse effects are not well characterized.

No information was identified on gastrointestinal effects in humans after oral exposure to chromium(III) compounds.

Oral exposure of animals to chromium(VI), but not chromium(III), compounds results in irritation and histopathological changes to tissues of the gastrointestinal tract. Gastrointestinal hemorrhage was observed in rats given a lethal gavage dose of potassium dichromate (130 mg chromium(VI)/kg) (Samitz 1970). Histopathological changes were observed in rats and mice exposed to chromium(VI) as sodium dichromate dihydrate in drinking water for 3 months (NTP 2007) or 2 years (NTP 2008a). Following exposure for 3 months, duodenal histiocytic infiltration of the duodenum was observed in male and female F344/N rats exposed at ≥3.5 mg chromium(VI)/kg/day. At the highest daily dose (20.9 mg chromium(VI)/kg/day), ulcer and epithelial hyperplasia and metaplasia of the glandular stomach were observed. Epithelial hyperplasia and histiocytic cellular infiltration of the duodenum was observed at ≥3.1 and ≥5.2 mg chromium(VI)/kg/day, respectively, in male and female B6C3F1 mice. Similar nonneoplastic lesions of the duodenum were also reported in the 3-month comparative study in male B6C3F1, BALB/c, and C57BL/6 mice, with epithelial hyperplasia at ≥2.8 mg chromium(VI)/kg/day in B6C3F1 and BALB/c strains and ≥5.2 in the C57BL/6 strain, and histiocytic cellular infiltration at ≥2.8 mg chromium(VI)/kg/day in B6C3F1 and C57BL/6 strains and ≥5.2 mg chromium(VI)/kg/day in the BALB/c strain. After exposure for 2 years, duodenal histiocytic infiltration was observed in male and female rats exposed at 0.77 and 2.4 chromium(VI)/kg/day, respectively; in mice, duodenal epithelial hyperplasia was observed at 0.38 mg chromium(VI)/kg/day for 2 years and histiocytic cellular infiltration of the duodenum was also observed in males at 2.4 mg chromium(VI)/kg/day and females at 3.1 mg chromium(VI)/kg/day. In the 2-year study (NTP 2008a), neoplasms of the squamous epithelium of the

oral mucosa and tongue were observed in rats and of the small intestine (duodenum, jejunum and ileum) were observed in mice; these findings are discussed in Section 3.2.2.7 (Oral Exposure, Cancer). In female mice exposed to 0.38 mg chromium(VI)/kg/day and male mice exposed to 2.4 mg chromium(VI)/kg/day for 2 years, cytoplasmic alteration of the pancreas (depletion of cytoplasm zymogen granules) was observed; NTP stated that the biological significance of this finding was uncertain (NTP 2008a). In contrast to the findings in the NTP 3-month and 2-year drinking water studies of sodium dichromate dihydrate (NTP 2007, 2008a), no histopathological changes to the gastrointestinal tract were observed in BALB/c mice exposed to dietary potassium dichromate at doses up to 36.7 chromium(VI)/kg/day in a multigeneration continuous breeding study (NTP 1997). Differences in results of these studies could be attributed to difference in the exposure media (water versus feed). Data from the 2-year drinking water study on sodium dichromate dihydrate in mice (NTP 2008a) were used to used to develop the chronic-duration oral MRL for chromium(VI) compounds. The $BMDL_{10}$ value of 0.09 mg chromium(VI)/kg/day for diffuse epithelial hyperplasia of the duodenum in female mice was used to calculate an oral MRL of 0.001 mg chromium(VI)/kg/day for chronic-duration exposure to chromium(VI) a compounds as described in the footnote of Table 3-3.

No histopathological changes to the stomach or small intestine were observed in mice and rats exposed to oral chromium(III) (as chromium nicotinate, chromium oxide, and chromium picolinate) for 3 months or 2 years (Ivankovic and Preussmann 1975; NTP 2008b; Rhodes et al. 2005; Shara et al. 2005, 2007). The highest doses of chromium(III) tested were 1,415 mg chromium(III)/kg/day as chromium picolinate in the diet for 3 months (NTP 2008b; Rhodes et al. 2005) and 2,040 mg chromium(III)/kg/day as chromium oxide in the diet 5 days/week for 2 years (Ivankovic and Preussmann 1975).

Hematological Effects. Cases of hematological effects have been reported in humans after the ingestion of lethal or sublethal doses of chromium(VI) compounds. In a case of an 18-year-old woman who ingested a few grams of potassium dichromate, decreased hemoglobin content and hematocrit, and increased total white blood cell counts, reticulocyte counts, and plasma hemoglobin were found 4 days after ingestion. These effects were indicative of intravascular hemolysis (Sharma et al. 1978). A 25-year-old woman who drank a solution containing potassium dichromate had a clinically significant increase in leukocytes due to a rise in polymorphonuclear cells (Goldman and Karotkin 1935). In another study, a 44-year-old man had decreased hemoglobin levels 9 days after ingestion of 4.1 mg chromium(VI)/kg as chromic acid solution that probably resulted from gastrointestinal hemorrhage (Saryan and Reedy 1988). Inhibition of blood coagulation was described in a case of a 17-year-old male who died after ingesting ~29 mg chromium(VI)/kg as potassium dichromate (Clochesy 1984; Iserson et al. 1983). Anemia

following severe hemorrhaging developed in a chrome plating worker who had accidentally swallowed an unreported volume of a plating fluid containing 300 g chromium trioxide/L. He was treated by hemodialysis, which saved his life (Fristedt et al. 1965).

In a cross-sectional study conducted in 1965 of 155 villagers whose well water contained 20 mg chromium(VI)/L as a result of pollution from an alloy plant in the People's Republic of China, associations were found between drinking the contaminated water and leukocytosis and immature neutrophils. The alloy plant began chromium smelting in 1961 and began regular production in 1965. Similar results were found in two similar studies in other villages, but further details were not provided (Zhang and Li 1987). The highest concentration of chromium(VI) detected during sampling was 20 mg chromium(VI)/L, equivalent to a dose of 0.57 mg chromium(VI)/kg/day. However, exposure estimates for this population are uncertain and it is likely that exposure levels in many cases were to concentrations <20 mg chromium(VI)/L. At least some residents obtained drinking water from alternative sources (Sedman et al. 2006) and exposure may have been self-limiting due to lack of palatability of water at higher concentrations (Beaumont et al. 2008). Thus, exposure levels associated with adverse effects are not well characterized.

No reliable information was identified on hematological effects in humans of oral exposure to chromium(III) compounds.

Microcytic, hypochromic anemia, characterized by decreased mean cell volume (MCV), mean corpuscular hemoglobin (MCH), hematocrit (Hct), and hemoglobin (Hgb), was observed in F344/N rats and B6C3F1 mice exposed to chromium(III) compounds in drinking water for exposure durations ranging from 4 days to 1 year (NTP 2007, 2008a). Severity was dose-dependent. Maximum effects were observed after approximately 3 weeks of exposure; with increasing exposures durations (e.g., 14 weeks to 1 year), effects were less pronounced, presumably due to compensatory hematopoietic responses. In general, effects were more severe in rats than mice. Following acute exposure of male rats to sodium dichromate dihydrate in drinking water for 4 days, a slight, but statistically significant decrease (2.1%) in MCH was observed at 2.7 mg chromium(VI)/kg/day, but not at 0.7 mg chromium(VI)/kg/day. With increasing doses (7.4 mg chromium(VI)/kg/day and greater), additional decreases in MCH and decreased MCV were observed (NTP 2008a). Similar effects were observed in male and female rats exposed for 5 days, with effects observed at 4.0 and 4.1 chromium(VI)/kg/day, respectively (NTP 2007); a NOAEL was not established.

More severe microcytic, hypochromic anemia occurred in rats and mice following exposure to sodium dichromate dihydrate in drinking water for 22 or 23 days (NTP 2007, 2008a). Decreased Hct (6.1%), Hgb (8.4%), MCV (7.7%), and MCH (10.6%) occurred in male rats exposed for 22 days to 0.77 mg chromium(VI)/kg/day, with decreases exhibiting dose-dependence; effects were not observed at 0.21 mg chromium(VI)/kg/day (NTP 2008a). Similar hematological effects were observed in male and female rats exposed to 1.7 mg chromium(VI)/kg/day for 23 days (NTP 2007). In female mice exposed to 22 days, slight, but significant decreases in MCV (2.0%) and MCH (1.2%) were observed at 0.38 mg chromium(VI)/kg/day, with more severe effects at higher doses (NTP 2008a). After exposure for 3 months to 1 year, microcytic, hypochromic anemia in rats and mice was less severe than that observed after 22 or 23 days (NTP 2007, 2008a). For example in male rats exposed for 22 days, decreases in Hct (6.1%), Hgb (8.4%), MCV (7.7%), and MCH (10.6%) were observed at 0.77 mg chromium(VI)/kg/day, whereas after exposure to 0.77 mg chromium(VI)/kg/day for 1 year, decreased MCH (2.4%), but not MCV, Hct ,or Hgb, were observed (NTP 2008a). Similar decreases in severity was also observed in female rats and in male and female mice exposed for 1 year compared to 22 days (NTP 2008a). In contrast, routine hematological examination revealed no changes in Sprague-Dawley rats exposed to 3.6 mg chromium(VI)/kg/day as potassium chromate in the drinking water for 1 year (MacKenzie et al. 1958); however, data on hematological parameters or statistical analyses were not presented in the report. Data from the 22-day evaluation in the 2-year NTP (2008a) drinking water study on sodium dichromate dihydrate in rats were used to used to develop the intermediate-duration oral MRL for chromium(VI) compounds. Because several hematological parameters are used to define the clinical picture of anemia, the intermediate-duration oral MRL was based on the average $BMDL_{2sd}$ value (e.g., the average of $BMDL_{2sd}$ values for Hgb, MCV, and MCH; BMD models did not provide adequate fit for hematocrit) of 0.52 mg chromium(VI)/kg/day, as described in the footnote of Table 3-3.

In feeding studies of potassium dichromate in Sprague-Dawley rats and BALB/c mice, slight microcytic hyprochromic anemia, characterized by slightly reduced MCV and MCH values was observed (NTP 1996a, 1996b, 1997). In rats and mice fed potassium dichromate for 9 weeks, MCV and MCH values, were decreased at the highest concentration only, which was equivalent to 8.4 and 9.8 mg chromium(VI)/ kg/day in male and female rats, respectively (NTP 1996b), and 32.2 and 48 mg chromium(VI)/kg/day in male and female mice, respectively (NTP 1996a). These effects did not occur at lower dietary concentrations equivalent to ≤2.1 or ≤2.45 mg chromium(VI)/kg/day for male and female rats, respectively, or to ≤7.35 or ≤12 mg chromium(VI)/day for male and female mice, respectively. In a multigeneration study of mice given potassium dichromate in the diet, F_1 males had decreased MCVs at dietary concentrations equivalent to 16 and 36.7 mg chromium(VI)/kg/day and decreased MCH values at

36.7 mg chromium(VI)/kg/day (NTP 1997). F_1 females had dose-related decreased MCV at concentrations equivalent to ≥7.8 mg chromium(VI)/kg/day. Since 7.8 mg chromium(VI)/kg/day was the lowest dose in the study, a no effect level was not identified. Compared to results of the drinking water studies on sodium dichromate dihydrate (NTP 2007, 2008a), hematological effects observed in the dietary studies on potassium dichromate (NTP 1996a, 1996b, 1997) occurred at higher daily doses. Differences may be related to differences in the exposure media (feed versus drinking water).

No hematological effects were observed in animals after oral exposure to chromium(III) compounds for exposure durations ranging from acute to chronic. Exposure of F344/N rats to chromium picolinate in the diet for 3 days at doses up to 506 mg chromium(III)/kg/day did not produce hematological effects (NTP 2008b). For intermediate duration exposure, no hematological effects were observed in rats exposed to chromic oxide in the diet at doses up to 1,806 chromium(III)/kg/day for 3 months (Ivankovic and Preussmann 1975), in rats and mice exposed to chromium picolinate in the diet at 506 and 1,415 mg chromium(III)/kg/day, respectively, for 3 months (NTP 2008b), or in rats chromium nicotinate in the diet at 1.5 or 0.25 mg chromium(III)/kg/day for 3 months or 38 weeks, respectively (Shara et al. 2005). For chronic exposure durations, no hematological abnormalities were found in rats exposed to 3.6 mg chromium(III)/kg/day as chromium trichloride in the drinking water for 1 year (MacKenzie et al. 1958), or in rats exposed to 0.25 mg chromium(III)/kg/day as chromium nicotinate for 2 years (Shara et al. 2007).

Musculoskeletal Effects. No information regarding musculoskeletal effects in humans exposed to oral chromium (VI) compounds was identified. The development of rhabdomyolysis was reported in a 24-year-old woman who ingested a dietary supplements containing chromium(III) picolinate (Martin and Fuller 1998). Over a 48-hour period, the patient ingested 1,200 μg of chromium(III) picolinate, equivalent to148.8 μg of chromium(III) or 2.2 μg of chromium(III)/kg body weight (based on a reported body weight of 67 kg) over a 48-hour period. Upon evaluation 4 days after initially ingesting the dietary supplement, she reported muscle pain on palpation and had muscular hypertrophy and elevated serum creatine kinase, although no myoglobin was detected in urine. In addition to chromium(III) picolinate, the dietary supplements contained numerous other substances.

Increases in serum creatine kinase (CK) activity were observed in F344/N rats following acute and intermediate exposure to sodium dichromate dihydrate in drinking water (NTP 2007). After exposure for 5 days, serum CK activity was increased in males by 31% at 31.8 mg chromium(VI)/kg/day and in females by 46% at 16.4 chromium(VI)/kg/day; after exposure for 13 weeks, serum CK activity was

increased by 70% and 50% in males and females, respectively, at 5.9 mg chromium(VI)/kg/day. Since serum CK activity increased with dose, NTP (2007) suggested that findings were consistent with muscle injury. After exposure of rats for 12 months to 2.4 mg chromium(VI)/kg/day as sodium dichromate dihydrate in drinking water, serum CK activity was increased by 64% (NTP 2008a). No information regarding muscoskeletal effects in animals exposed to oral chromium(III) compounds was identified.

Hepatic Effects. Effects on the liver have been described in case reports of humans who had ingested chromium(VI) compounds. Liver damage, evidenced by the development of jaundice, increased bilirubin, and increased serum lactic dehydrogenase, was described in a case of a chrome plating worker who had accidentally swallowed an unreported volume of a plating fluid containing 300 g chromium trioxide/L (Fristedt et al. 1965). In a 14-year-old boy who died after ingesting 7.5 mg chromium(VI)/kg as potassium dichromate, high levels of the liver enzymes, glutamic-oxaloacetic transaminase (aspartate aminotransferase) and glutamic-pyruvic transaminase (alanine aminotransferase), were found in the serum 24 hours after ingestion. Upon postmortem examination, the liver had marked necrosis (Kaufman et al. 1970). Fatty degeneration of the liver was observed on autopsy of a 35-year-old female who died after ingesting approximately 257 mg chromium(VI)/kg (assuming a70-kg body weight) as chromic acid in a suicide (Loubieres et al. 1999).

Effects on the liver of rats and mice exposed to oral chromium(VI) compounds for acute, intermediate and chronic durations have been detected by biochemical and histochemical techniques. In male and female F344/N rats exposed to 4.0 and 4.1 mg chromium(VI)/kg/day, respectively, as disodium dichromate in drinking water for 5 days, serum alanine aminotransferase (ALT) activity was increased by 15 and 30%, respectively (NTP 2007). After 14 weeks of exposure, serum ALT activity was increased by 14% in male rats and by 30% in female rats and serum sorbital dehydrogenase (SDH) activity was increased by 77% in male rats and 359% in female rats at 1.7 mg chromium(VI)/kg/day (NTP 2007). In females, morphological changes to the liver included cellular histiocyte infiltration and chronic focal inflammation at doses of 3.5 and 20.9 mg chromium(VI)/kg/day, respectively; no morphological changes were observed in male rats, indicating that female rats may be more sensitive than males. However, similar exposure to B6C3F1 mice to 27.9 mg chromium(VI)/kg/day for 14 weeks produced no effects on serum liver enzymes or hepatic morphology (NTP 2007). Increased serum ALT and aspartate aminotransferase (AST) activities and hepatic morphological changes (vacuolization, increased sinusoidal space, and necrosis) were observed in rats exposed to 1.3 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 22 weeks (Acharya et al. 2001). Increased serum ALT (253%) and histopathological changes (focal necrosis and degeneration with changes in vascularization) were reported

in Wistar rats exposed to chromium(VI) (compound not specified) in drinking water for 10 weeks (Rafael et al. 2007). Rats treated by gavage with 13.5 mg chromium(VI)/kg/day as potassium chromate for 20 days had increased accumulations of lipids (Kumar and Rana 1982) and changes and relocalization of liver enzymes (alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, cholinesterase, and lipase) (Kumar et al. 1985), as determined by histochemical means. In another study, no treatment-related histological changes in liver cells were observed in groups of Sprague-Dawley rats containing 24 males and 48 females that were exposed to chromium(VI) as potassium dichromate in the diet for 9 weeks followed by a recovery period of 8 weeks (NTP 1996b). Average daily ingestion of chromium(VI) for males was 1, 3, 6, and 24 mg/kg/day and 1, 3, 7, and 28 mg/kg/day for females. Although no indication of hepatic effects was found in mice exposed to ≤36.7 mg/kg/day in a multigeneration feeding study (NTP 1997), some indication of liver toxicity was found in a 9-week feeding study in BALB/c mice exposed to 1.1, 3.5, 7.4, and 32 mg chromium(VI)/kg/day for males and 1.8, 5.6, 12, and 48 mg chromium(VI)/kg/day for females (NTP 1996a). Hepatocyte cytoplasmic vacuolization occurred in 1/6 males at 3.5 mg/kg/day, 2/5 males at 7.4 mg/kg/day, and 2/6 males at 32 mg/kg/day, and in 1/12 control females, 0/12 females at 1.8 mg/kg/day, 3/12 females at 5.6 mg/kg/day, 2/12 females at 12 mg/kg/day, and 4/12 females at 48 mg/kg/day. The vacuoles were small, clear, and well demarcated, which is suggestive of lipid accumulation. The small number of animals and lack of a clear dose-response preclude a definitive conclusion as to whether this effect was toxicologically significant. For chronic exposure durations, adverse liver effects have been observed in F344/N rats and B6C3F1 mice exposed to chromium(VI) as sodium chromate dihydrate in drinking water (NTP 2008a). In male rats exposed for 1 year to 0.77 mg chromium(VI)/kg/day, serum ALT activity was increased by 156%. After exposure for 2 years, histopathological examination of the liver showed the following morphological changes, with females of both species appearing more sensitive than males: chronic inflammation (2.1 chromium(VI)/kg/day), histiocytic cellular infiltration (5.9 chromium(VI)/kg/day) and basophilic foci (0.77 chromium(VI)/kg/day) in male rats; chronic inflammation (0.24 mg chromium(VI)/kg/day), histiocytic cellular infiltration (0.94 mg chromium(VI)/kg/day) and fatty change (0.94 mg chromium(VI)/kg/day) in female rats; clear cell and eosinophilic foci in male mice (5.9 mg chromium(VI)/kg/day); and histiocytic cellular infiltration (0.38 mg chromium(VI)/kg/day) and chronic inflammation (3.1 mg chromium(VI)/kg/day) in female mice (NTP 2008a). No morphological changes, however, were detected in the livers of rats exposed to 3.6 mg chromium(VI)/kg/day as potassium chromate in the drinking water for 1 year (MacKenzie et al. 1958).

No evidence of liver damage has been observed in rats and mice treated with oral chromium(III) compounds for intermediate and chronic exposure durations, based on histopathological examination of

the liver. For intermediate-duration exposures, no morphological changes were observed in rats exposed to 1,806 mg chromium(III)/kg/day as chromium oxide in the diet 5 days/week for 90 days (Ivankovic and Preussmann 1975), rats exposed to 9 mg chromium(III)/kg/day as chromium chloride or chromium picolinate in the diet for 20 weeks (Anderson et al. 1997b), rats exposed to 506 mg chromium(III)/kg/day and mice exposed to 1,415 mg chromium(III)/kg/day as chromium picolinate in the diet for 14 weeks (NTP 2008b; Rhodes et al. 2005), or rats exposed to chromium nicotinate in the diet at 1.5 mg chromium(III)/kg/day for 14 weeks or 0.25 mg chromium(III)/kg/day as chromium nicotinate for 38 weeks (Shara et al. 2005, 2007). For chronic-duration exposures, histological examination revealed no morphological changes in the livers of rats exposed to chromium oxide in the diet 5 days/week at 2,040 mg chromium(III)/kg/day for 2 years (Ivankovic and Preussmann 1975), rats exposed to 3.6 mg chromium(III)/kg/day as chromium trichloride in the drinking water for 1 year (MacKenzie et al. 1958), of rats exposed to 513 mg chromium(III)/kg/day and mice exposed to 781 mg chromium(III)/kg/day as chromium picolinate in the diet for 2 years (NTP 2008b), rats exposed to 0.25 mg chromium(III)/kg/day as chromium nicotinate in the diet for 2 years (Shara et al. 2005, 2007), or rats exposed to 0.46 mg chromium(III)/kg/day as chromium acetate in the drinking water for 2–3 years (Schroeder et al. 1965).

Renal Effects. Case studies were located regarding renal effects in humans after oral exposure to chromium(VI) compounds. Acute renal failure, characterized by proteinuria, and hematuria, and followed by anuria, developed in a chrome plating worker who had accidentally swallowed an unreported volume of a plating fluid containing 300 g chromium trioxide/L. He was treated by hemodialysis (Fristedt et al. 1965). Necrosis of renal tubules was found upon autopsy of a 22-month-old boy who died after ingesting an unknown amount of sodium dichromate (Ellis et al. 1982) and of a 17-year-old boy who died after ingesting 29 mg chromium(VI)/kg as potassium dichromate (Clochesy 1984; Iserson et al. 1983). A fatal ingestion of 4.1 mg chromium(VI)/kg as a chromic acid solution in a 44-year-old man resulted in acute tubular necrosis and renal failure (Saryan and Reedy 1988). A 14-year-old boy who ingested 7.5 mg chromium(VI)/kg as potassium dichromate died from renal failure 8 days after he was admitted to the hospital. Upon postmortem examination, the kidneys were pale, enlarged, and necrotic with tubular necrosis and edema (Kaufman et al. 1970). Acute renal failure and necrosis also observed on autopsy of a 35-year-old woman who died following ingestion of 357 mg chromium(VI)/kg as chromic acid (Loubieres et al. 1999). Another case study of an 18-year-old woman who ingested a few grams of potassium dichromate reported proteinuria, oliguria, and destruction of the tubular epithelium of the kidneys. She regained renal function following dialysis (Sharma et al. 1978). Proteinuria and oliguria were also observed after ingestion of potassium dichromate by a 25-year-old woman (Goldman and Karotkin 1935).

Acute renal failure was reported in a 24-year-old man who ingested the an unknown quantity of a dietary supplement (Arsenal X®) containing chromium picolinate daily for 2 weeks (Wani et al. 2006). Serum creatinine was elevated approximately 3 times above the normal range, blood urea nitrogen was elevated slightly above normal range, urinalysis was positive for protein, and renal biopsy showed acute tubular necrosis. The patient developed severe impairment of renal function that required hemodialysis. Renal function improved within 4 weeks of discontinuation of treatment with the supplement. Chemical analysis of the dietary supplement was not conducted and the patient's plasma chromium levels were not obtained. Adverse renal effects were reported in a 49-year-old woman who ingested 600 µg of chromium(III) picolinate (equivalent to 74.4 µg chromium(III)/day or 1.1 µg chromium(III)/kg/day, assuming a body weight of 70 kg) daily for 6 weeks (Wasser et al. 1997). The patient was evaluated approximately 5 months after initiating the 6-week treatment. Serum creatinine levels were approximately 6 times above the normal range, blood urea nitrogen was approximately 4 times above the normal range, and trace amounts of blood were found in the urine. Renal biopsy showed severe chronic active interstitial nephritis. After 2 months of treatment with prednisone, serum creatinine levels were approximately 4 times above the normal range (other values were not reported) Chemical analysis of the dietary supplement was not conducted and the patient's plasma chromium levels were not obtained.

Renal effects have been observed in animals following oral exposure to chromium(VI), but not chromium(III), compounds. Effects on the kidneys of rats exposed to potassium chromate have been detected by biochemical and histochemical techniques. Rats treated by gavage with 13.5 mg chromium(VI)/kg/day for 20 days had increased accumulation of lipids and accumulated triglycerides and phospholipids in different regions of the kidney than controls (Kumar and Rana 1982). Similar treatment of rats also resulted in inhibition of membrane and lysosomal enzymes (alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, and lipase) in the kidneys (Kumar and Rana 1984). Histopathological changes to the kidneys, including vacuolization in glomeruli, degeneration of basement membrane of Bowman's capsule, and renal tubular epithelial degeneration, were observed in Wistar rats exposed to 1.3 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 22 weeks (Acharya et al. 2001). Oliguria and proteinuria were observed in Wistar rats exposed to 100 mg chromium(VI)/kg/day as sodium chromate in drinking water for 28 days (Diaz-Mayans et al. 1986). However, histological examination revealed no morphological changes in the kidneys of rats exposed to 3.6 mg chromium(VI)/kg/day as potassium chromate in drinking water for 1 year (MacKenzie et al. 1958). Results of studies in rats and mice conducted by NTP (1996a, 1996b, 1997, 2007, 2008a) also show no histopathological changes in kidneys following intermediate-or chronic-duration exposure to

chromium(VI) compounds in the diet or drinking water. The respective highest doses of chromium(VI) tested for intermediate and chronic exposure durations were 48 mg chromium(VI)/kg/day in mice exposed to dietary potassium dichromate for 9 weeks (NTP 1996a) and 8.7 chromium(VI)/kg/day, as sodium dichromate dihydrate in drinking water (NTP 2007, 2008a).

Exposure of mice and rats to chromium(III) compounds (chromium acetate, chromium nicotinate, chromium oxide, chromium picolinate, and chromium trichloride) in food or drinking water for up to 2 years did not result in renal damage, based on histopathological examination of kidneys (Anderson et al. 1997b; Ivankovic and Preussmann 1975; MacKenzie et al. 1958; NTP 2008b; Schroeder et al. 1965; Shara et al. 2005, 2007). The respective highest doses of chromium(III) tested for intermediate and chronic exposure durations were 1,806 mg chromium(III)/kg/day as chromium oxide in the diet 5 days/week for 3 months and 2,040 mg chromium(III)/kg/day chromium oxide in the diet 5 days/week for 2 years (Ivankovic and Preussmann 1975). Renal function was not assessed in these studies.

Endocrine Effects. No studies were located regarding endocrine effects in humans following oral exposure to chromium(VI) or (III) compounds. Serum prolactin levels were decreased by 59% in male Wistar rats exposed to 74 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 30 days (Quinteros et al. 2007). Histopathological examination of the endocrine tissues (including adrenal gland, parathyroid, and thyroid) has been evaluated in rats and mice exposed to oral chromium(VI) (as sodium dichromate dihydrate) and chromium(III) (as chromium nicotinate and chromium picolinate) for durations of 3 months to 2 years, with no abnormalities observed (NTP 2007, 2008a, 2008b; Shara et al. 2005, 2007). For chromium(VI) compounds, the highest doses tested for intermediate and chronic exposure durations were 27.9 and 8.7 chromium(VI)/kg/day, respectively, as sodium dichromate dihydrate in drinking water (NTP 2007, 2008a). For chromium(III) compounds, the highest doses tested for intermediate and chronic exposure durations were 1,415 and 781 mg chromium(III)/kg/day, respectively, as chromium picolinate in the diet for 3 months and 2 years, respectively (NTP 2008b; Rhodes et al. 2005). Endocrine function was not assessed in these studies.

Dermal Effects. Administration of 0.04 mg chromium(VI)/kg as potassium dichromate in an oral tolerance test exacerbated the dermatitis of a building worker who had a 20-year history of chromium contact dermatitis. A double dose led to dyshidrotic lesions (vesicular eruptions) on the hands (Goitre et al. 1982). Dermatitis in 11 of 31 chromium-sensitive individuals worsened after ingestion of 0.036 mg chromium(VI)/kg as potassium dichromate (Kaaber and Veien 1977). The sensitizing exposures were not

discussed or quantified. No information regarding dermal effects of oral exposure of humans to chromium(III) compounds was identified.

No studies were located regarding noncancer dermal effects in animals after oral exposure to chromium(VI) or chromium(III) compounds. The effect of oral exposure to chromium(VI) compounds on increased susceptibility of hairless mice to ultraviolet light-induced skin cancer is discussed in Section 3.2.2.7 (Oral Exposure, Cancer).

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to chromium(VI) or chromium(III) compounds. Histopathologic examination of rats and mice exposed to sodium dichromate dihydrate in drinking water at 20.9 and 27.9 mg chromium(IV)/kg/day, respectively, for 3 months or at 7.0 and 8.7 mg chromium(IV)/kg/day, respectively, for 2 years revealed normal morphology of the ocular tissue (NTP 2007, 2008a). Similar negative findings were observed in rats and mice exposed to chromium(III) as dietary chromium picolinate at 506 and 1415 mg chromium(III)/kg/day, respectively, for 3 months or at 313 and 781 mg chromium(III)/kg/day, respectively, for 2 years (NTP 2008b).

Body Weight Effects. Studies reporting body weight effects in humans exposed to chromium(VI) were not identified. The potential beneficial effect of dietary supplementation with chromium(III) (as chromium picolinate or other chromium(III) compounds) to aid in weight loss and increase lean body mass has been reported. Although the role of chromium(III) in the regulation of lean body mass, percentage body fat, and weight reduction is highly controversial with negative and positive results being reported in the literature, studies assessing these effects were not designed to evaluate weight loss as a toxicological end point (Anderson 1998b). Thus, body weight effects associated with dietary supplementation with chromium(III) compounds is not considered adverse (see Section 2.2 for additional information).

Significant decreases in body weight have been reported in several intermediate-duration oral chromium(VI) studies in animals (Bataineh et al. 1997; Chowdhury and Mitra 1995; De Flora et al. 2006; Elbetieha and Al-Hamood 1997; NTP 1996a, 1996b, 2007; Quinteros et al. 2007; Yousef et al. 2006). However, it should be noted that high concentrations of chromium in drinking water decrease palatability of water, resulting in decreased water consumption; thus, decreased body weight may, in part, be due to decreased water consumption, in addition to other causes. In male rats exposed to 73 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 30 days, body weight was decreased

by 11.6% (Quinteros et al. 2007). A 19% decrease in body weight gain was observed male rats exposed to 42 mg chromium(VI)/kg/day (Bataineh et al. 1997) and a 10% decrease was reported in male mice exposed to 6 mg chromium(VI)/kg/day (Elbetieha and Al-Hamood 1997) as potassium dichromate in drinking water for 12 weeks. Note that daily doses in the study by Elbetieha and Al-Hamood (1997) may be overestimated, due to decreased water consumption in the higher concentration group (decrease was not quantified by study authors). Final body weight was decreased in rats and mice exposed to sodium dichromate dihydrate in drinking water for 14 weeks (NTP 2007). In rats, body weight was decreased in males by 11% at 11.2 mg chromium(VI)/kg/day and in females by 6% at 20.9 mg chromium(VI)/kg/day; in mice, body weight was decreased by 6% in males at 3.1 mg chromium(VI)/kg/day and by 8% in females at 5.2 mg chromium(VI)/kg/day. Decreases in body weight were also observed in male mice (9.3%) and female (13.5%) mice exposed to 165 and 14 mg chromium(VI)/kg/day as sodium dichromate dihydrate in drinking water for 210 days (De Flora et al. 2006). Gavage administration of 40 or 60 mg chromium(VI)/kg/day as sodium dichromate for 90 days resulted in 57 and 59% decreases in body weight gain, respectively (Chowdhury and Mitra 1995). In contrast, no changes in body weight gain were seen in rats or mice exposed to 9.8 or 48 mg chromium(VI)/kg/day, respectively, as potassium dichromate in the diet for 9 weeks (NTP 1996a, 1996b) or in rabbits administered 3.6 mg chromium(VI)/kg/day by gavage as potassium dichromate (Yousef et al. 2006). No alterations in body weight gain were observed in rats chronically exposed (1 year) to 3.6 mg chromium(VI)/kg/day as potassium chromate in drinking water (Mackenzie et al. 1958). In contrast, final body weight was decreased by 12% decrease male rats at 5.9 mg chromium(VI)/kg/day and by 11% in female rats at 7.0 mg chromium(VI)/kg/day as sodium dichromate dihydrate in drinking water for 2 years (NTP 2008a).

Several studies have examined the effect of exposure to potassium dichromate in drinking water on maternal body weight gain. An acute exposure (9 days) resulted in 8 and 24% decreases in body weight gain in pregnant mice exposed to 101 or 152 mg chromium(VI)/kg/day, respectively (Junaid et al. 1996b). Similarly, a decrease in maternal body weight gain was observed in pregnant mice exposed to 98 mg chromium(VI)/kg/day as potassium dichromate for 19 days (Trivedi et al. 1989). Reduced maternal body weight gains of 8, 14, and 21% were observed in rats exposed to 37, 70, or 87 mg chromium(VI)/kg/day for 20 days prior to mating (Kanojia et al. 1996). Similar decreases in body weight gain (18 and 24%) were observed in rats exposed to 89 or 124 mg chromium(VI)/kg/day, respectively, for 3 months prior to mating (Kanojia et al. 1998). However, no alterations in maternal body weight gain were observed in a continuous breeding study in which rats were exposed to 36.7 mg chromium(VI)/kg/day as potassium dichromate in the diet (NTP 1997).

Conflicting results have been reported for alterations in body weight in rats and mice exposed to oral chromium(III) compounds for intermediate and chronic exposure durations. Dietary exposure to 9 mg chromium(III)/kg/day as chromium chloride or chromium picolinate for 20 weeks (Anderson et al. 1997b) or 3.6 mg chromium(III)/kg/day as chromium chloride (Mackenzie et al. 1958) did not result in significant alterations in body weight gain. No alterations on body weight were observed in rats or mice exposed to dietary chromium picolinate for 14 weeks at doses up to 506 and 1,415 mg chromium(III)/kg/day, respectively (NTP 2008b; Rhodes et al. 2005) or in male and female mice exposed to chromic potassium sulfate in drinking water for 210 days at doses of 165 and 140 mg chromium(III)/kg/day, respectively (De Flora et al. 2006). No change in body weight was observed in male and female rats exposed to dietary chromium nicotinate for 90 days at 1.5 and 1.2 mg chromium(III)/kg/day, respectively (Shara et al. 2005); however, body weight was decreased by 8.1% in males at 0.22 mg chromium(III)/kg/day and by 11.4% in females at 0.25 mg chromium(III)/kg/day following exposure to dietary chromium nicotinate for 38 weeks (Shara et al. 2007). Exposure to chromium chloride in drinking water resulted in 14 and 24% decreases in body weight gain in rats exposed to 40 mg chromium(III)/kg/day for 12 weeks (Bataineh et al. 1997) and male mice exposed to 5 mg chromium(III)/kg/day for 12 weeks (Elbetieha and Al-Hamood 1997), respectively. Note that daily doses in the study by Elbetieha and Al-Hamood (1997) may be overestimated, due to decreased water consumption in the higher concentration group (decrease was not quantified by study authors). No alterations in body weight gain were observed in rats or mice exposed to 0.46 or 0.48 mg chromium(III)/kg/day, respectively, as chromium acetate for a lifetime (Schroeder et al. 1964, 1965), or in mice and rats exposed to dietary chromium picolinate for 2 years at doses up to 313 and 781 mg chromium(III)/kg/day, respectively (NTP 2008b). However, exposure to dietary chromium nicotinate for 2 years resulted in a 14.9% decrease in male rats at 0.22 mg chromium(III)/kg/day and a 9.6% decrease in female rats at 0.25 mg chromium(III)/kg/day (Shara et al. 2007).

Metabolic Effects. Metabolic acidosis was observed in a 35-year-old female died after ingesting approximately 257 mg chromium(VI)/kg (assuming a 70-kg body weight) as chromic acid in a suicide (Loubieres et al. 1999). No information on adverse metabolic effects of chromium(III) compounds in humans was identified. Serum glucose was elevated by 65% in male Wistar rats exposed to 3.7 mg chromium(VI)/kg/day (compound not specified) in drinking water for 10 weeks (Rafael et al. 2007). No changes in serum glucose were reported in rats and mice exposed to sodium dichromate dihydrate in drinking water for 3 months at doses up to 27.9 mg chromium(VI)/kg/day or for 2 years at doses up to 8.7 mg chromium(VI)/kg/day (NTP 2007, 2008a); however, data on serum glucose were not presented in

the study reports. No information on adverse metabolic effects of chromium(III) compounds in animals was identified.

3.2.2.3 Immunological and Lymphoreticular Effects

The only reported effect of orally exposed humans on the immune system was the exacerbation of chromium dermatitis in chromium-sensitive individuals, as noted for dermal effects in Section 3.2.2.2. Sensitization of workers, resulting in respiratory and dermal effects, has been reported in numerous occupational exposure studies. Although the route of exposure for initial sensitization in an occupational setting is most likely a combination of inhalation, oral, and dermal routes, information on the exposure levels producing sensitization by the oral route was not identified. Additional information on contact dermatitis in sensitized workers is provided in Section 3.2.3.3 (Dermal Exposure, Immunological and Lymphoreticular Effects).

Oral exposure of animals to chromium(VI), but not chromium (III), compounds resulted in functional and histopathological changes to the immune system (NTP 2007, 2008a; Snyder and Valle 1991). Splenocytes prepared from rats given potassium chromate in drinking water at 16 mg chromium(VI)/kg/day for 3 weeks showed an elevated proliferative response of T-and B-lymphocytes to the mitogens, concanavalin A and liposaccharide, compared with splenocytes from control rats. A 5-fold enhancement of the proliferative response to mitomycin C was also seen when splenocytes from rats exposed for 10 weeks were incubated with splenocytes from nonexposed rats and additional chromium (0.1 mg chromium(VI)/L) was added to the incubation compared to the system without added chromium. It was suggested that these increased proliferative responses represent chromium-induced sensitization (Snyder and Valle 1991). Microscopic changes to lymphatic tissues, including histiocytic cellular infiltration of mesenteric and/or pancreatic nodes, were observed in rats and mice exposed to sodium dichromate dihydrate in drinking water for 3 months or 2 years (NTP 2007, 2008a). Following 3 months of exposure, histiocytic cellular infiltration was observed in male and female rats at 1.7 and 20.9 mg chromium(VI)/kg/day, respectively, and in mice at 3.1 mg chromium(VI)/kg/day (NTP 2007). After 2 years of exposure, histiocytic cellular infiltration and hemorrhage of mesenteric lymph nodes were observed in male rats at 0.77 mg chromium(VI)/kg/day (NTP 2008a). Histiocytic cellular infiltration of lymph nodes, but not hemorrhage, was observed at 2.4 mg chromium(VI)/kg/day in female rats and at 0.38 mg chromium(VI)/kg/day in mice (NTP 2008a). No abnormal histopathological changes were observed in lymphatic tissues of rats and mice exposed to oral chromium(III) (as chromium nicotinate and chromium picolinate) for 3 months or 2 years (NTP 2008b; Rhodes et al. 2005; Shara et al. 2005, 2007).

These highest doses of chromium(III) tested for intermediate and chronic exposure durations were 1,415 mg chromium(III)/kg/day as chromium picolinate in feed for 3 months and 781 mg chromium(III)/kg/day as chromium picolinate in feed for 2 years. The NOAEL and LOAEL values are recorded in Table 3-3 and plotted in Figure 3-3 for chromium(VI) and recorded in Table 3-4 and plotted in Figure 3-4 for chromium(III).

3.2.2.4 Neurological Effects

The only information regarding neurological effects in humans after oral exposure to chromium(VI) is the report of an enlarged brain and cerebral edema upon autopsy of a 14-year-old boy who died after ingesting 7.5 mg chromium(VI)/kg as potassium dichromate. These effects may be the result of accompanying renal failure (Kaufman et al. 1970). No information was identified on neurological effects in humans after oral exposure to chromium(III) compounds.

A decrease in motor activity and balance was reported in rats given 98 mg chromium(VI)/kg/day as sodium chromate in drinking water for 28 days (Diaz-Mayans et al. 1986). No additional studies were identified evaluating neurological function in laboratory animals following oral exposure to chromium(VI) or chromium(III) compounds. Histopathological examination of the brain and nervous system tissues has been evaluated in rats and mice exposed to oral chromium(VI) (as sodium dichromate dihydate) and chromium(III) (as chromium nicotinate, chromium oxide, and chromium picolinate) for durations of 3 months to 2 years, with no abnormalities observed (Ivankovic and Preussmann 1975; NTP 2007, 2008a, 2008b; Shara et al. 2005, 2007). For chromium(VI) compounds, the highest doses tested for intermediate and chronic exposure durations were 27.9 and 8.7 chromium(VI)/kg/day, respectively, as sodium dichromate dihydrate in drinking water (NTP 2007, 2008a). For chromium(III) compounds, the highest doses tested for intermediate and chronic exposure durations were 1,806 and 2,040 mg chromium(III)/kg/day, respectively, as chromium oxide in the diet 5 days/week (Ivankovic and Preussmann 1975). None of theses studies conducted more sensitive neurological, neurochemical, or neurobehavioral tests.

The NOAEL and LOAEL values are recorded in Table 3-3 and plotted in Figure 3-3 for chromium(VI) and recorded in Table 3-4 and plotted in Figure 3-4 for chromium(III).

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to chromium(VI) or chromium(III) compounds.

A number of studies have reported reproductive effects in animals orally exposed to chromium(VI). Functional and morphological effects on male reproductive organs have been reported in monkeys, rats, mice, and rabbits. In a series of studies in male bonnet monkeys (*Macaca radiate*) (Aruldhas et al. 2004, 2005, 2006; Subramanian et al. 2006), decreased testes weight, histopathological changes of the epididymis, disrupted spermatogenesis, and decreased sperm count and motility were observed following exposure to 2.1, 4.1, and 8.3 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 180 days. Histopathological changes, characterized by ductal obstruction and development of microcanals, germ cell depletion, hyperplasia of Leydig cells, and Sertoli cell fibrosis, increased in severity with dose. Sperm count and motility were significantly decreased, with effects exhibiting duration- and dose-dependence (Subramanian et al. 2006). After exposure for 2 months, significant decreases in sperm count (by 13%) and motility (by 12%) were observed only in monkeys treated with 8.3 mg chromium(VI)/kg/day, whereas after 6 months, dose-dependent decreases in sperm count and motility were observed at doses of ≥2.1 mg chromium(VI)/kg/day. No effects on sperm count or motility were observed in monkeys treated with 1.1 chromium(VI)/kg/day, although histopathological assessment of male reproductive tissues was not conducted in this dose group.

Exposure of male Wistar rats to 5.2 and 10.4 mg chromium(VI)/kg/day administered as chromic acid by gavage for 6 days produced decreased sperm count and histopathological changes to the testes (Li et al. 2001). Similar effects occurred at both doses, with sperm count decreased by 75.5 and 79.6% at 5.2 and 10.4 mg chromium(VI)/kg/day, respectively, and the “level of abnormal sperm" was increased 2.4-fold and 2.8-fold at 5.2 and 10.4 mg chromium(VI)/kg/day, respectively. Histopathological assessment of testes showed decreased diameter of seminiferous tubules and germ cell rearrangement within the tubules. In contrast, exposure of F344/N male rats to chromium(VI) as sodium dichromate dihydrate in drinking water at doses up to 20.9 or mg chromium(VI)/kg/day for 3 months or 5.9 mg chromium(VI)/kg/day for 2 years did not produce histopathological changes to male reproductive tissues (NTP 2007, 2008a).

Male reproductive effects were observed in groups of 10 mature male Charles Foster strain rats administered 20, 40, and 60 mg chromium(VI)/kg/day as sodium dichromate(VI) by gavage for 90 days (Chowdhury and Mitra 1995). Testis weight, population of Leydig cells, seminiferous tubular diameter,

testicular protein, DNA, and RNA were all significantly reduced at 40 and 60 mg chromium(VI)/kg/day. The number of spermatogonia was not affected by treatment; however, resting spermatocytes (high dose), pachytene spermatocytes (high dose, intermediate dose) and stage-7 spermatid (high and intermediate doses) counts were significantly reduced and were treatment related. Testicular activity of succinic dehydrogenase was significantly lowered in the two high-dose groups, testicular cholesterol concen trations were elevated in the highest-dosed group, and both serum testosterone and testicular levels of 3β-Δ^5-hydroxysteroid dehydrogenase were significantly lowered. The authors also determined that the total testicular levels of ascorbic acid in the two higher-dosing groups was about twice that of the control values whereas, in the highest-treated group the total ascorbic acid levels were about half those of controls. At the low dose (20 mg/kg/day), testicular protein, 3β-Δ^5-hydroxysteroid dehydrogenase, and serum testosterone were decreased. The authors indicated that chromium enhanced levels of the vitamin, but at the highest dose, testicular levels became exhausted, thus decreasing the ability of the cells to reduce chromium(VI).

Significant alterations in sexual behavior and aggressive behavior were observed in male Sprague-Dawley rats exposed to 42 mg chromium(VI)/kg/day as potassium dichromate in the drinking water for 12 weeks (Bataineh et al. 1997). The alterations in sexual behavior included decreased number of mounts, lower percentage of ejaculating males, and increased ejaculatory latency and postejaculatory interval. The adverse effects on aggressive behavior included significant decreases in the number of lateralizations, boxing bouts, and fights with the stud male and ventral presenting. No significant alterations in fertility were observed when the exposed males were mated with unexposed females.

Reduced sperm count and degeneration of the outer cellular layer of the seminiferous tubules were observed in BALB/c mice exposed for 7 weeks to 15.2 mg chromium(VI)/kg/day as potassium dichromate in the diet (Zahid et al. 1990). Morphologically altered sperm occurred in mice given diets providing 28 mg chromium(VI)/kg/day as potassium dichromate. No effect was found on testis or epididymis weight, and reproduction function was not assessed. In contrast, an increase in testes weight was observed in Swiss mice exposed in drinking water to 6 mg chromium(VI)/kg/day as potassium dichromate for 12 weeks. At the next highest dose (14 mg chromium(VI)/kg/day), decreases in seminal vesicle and preputial gland weights were observed, although no information of sperm count was reported (Elbetieha and Al-Hamood 1997). At the higher exposure level, mice consumed less water (data on water consumption were not included in the study report); thus, the daily chromium(VI) dose may be overestimated for this exposure group. In studies designed to confirm or refute the findings of the Zahid et al. (1990) study, the reproductive effects of different concentrations of chromium(VI) as potassium

dichromate in the diet on BALB/c mice and Sprague-Dawley rats were investigated (NTP 1996a, 1996b). Groups of 24 of each species were fed potassium dichromate(VI) in their feed continuously for 9 weeks followed by an 8-week recovery period. For mice, the average daily ingestions of chromium(VI) were 1.05, 3.5, 7.5, and 32.2 mg/kg/day for males and for rats were 0.35, 1.05, 2.1, and 8.4 mg/kg/day (NTP 1996b). Microscopic examinations of the testes and epididymis for Sertoli nuclei and preleptotene spermatocyte counts in stage X or XI tubules did not reveal any treatment-related effects. Similarly, exposure to sodium dichromate dihydrate in drinking water did not produce morphological changes to male reproductive organs of B6C3F1 mice exposed to 27.9 or 5.9 mg chromium(VI)/kg/day for 3 months or 2 years, respectively, or affect sperm count or motility in male B6C3F1, BALB/c and C57BL/6N mice exposed to 8.7 mg chromium(VI)/kg/day for 3 months (NTP 2007, 2008a).

Reduced sperm count and plasma testosterone were observed in male New Zealand rabbits administered 3.6 mg chromium(VI)/kg/day as potassium dichromate for 10 weeks by gavage (Yousef et al. 2006). Sperm count was decreased by 18%, total sperm output was decreased by 25.9%, total number of mobile sperm was decreased by 34.3%, and number of dead sperm increased by 23.9%. In addition, relative weight of testes and epididymis were decreased by 22.2% and plasma testosterone was decreased by 20.8%.

Effects of chromium(VI) on the female reproductive system have been reported in rats and mice. Murthy et al. (1996) reported a number of reproductive effects in female Swiss albino mice exposed to potassium dichromate in drinking water for 20 days. The observed effects included a significant reduction in the number of follicles at different stages of maturation at ≥60 mg chromium(VI)/kg/day, reduction in the number of ova/mice at ≥120 mg chromium(VI)/kg/day, significant increase in estrus cycle duration at 180 mg chromium(VI)/kg/day, and histological alterations in the ovaries (e.g., proliferated, dilated, and congested blood vessels, pyknotic nuclei in follicular cells, and atretic follicles) at ≥120 mg chromium(VI)/kg/day. The severity of the reproductive effects appeared to be dose-related. In an ancillary study, electron microscopy of selected ovarian tissues revealed ultrastructural changes (disintegrated cell membranes of two-layered follicular cells and altered villiform cristae of mitochondria and decreased lipid droplets in interstitial cells) in mice exposed to 1.2 mg chromium(VI)/kg/day for 90 days; the toxicological significance of these alterations is not known. The study authors suggest that the effects observed in the interstitial cells may be due to a reduction in lipid synthesizing ability, which could lead to decreased steroid hormone production. An increase in relative ovarian weight was observed in female Swiss mice exposed for 12 weeks to 14 mg chromium(VI)/kg/day as potassium dichromate (Elbetieha and Al-Hamood 1997) , although the calculated daily dose may be overestimated, due to

decreased water consumption in the higher concentration group (decrease was not quantified by study authors). In contrast, microscopic examinations of the ovaries showed no treatment-related effects in female BALB/c mice and Sprague-Dawley rats fed up to 9.8 and 48 mg chromium(VI)/kg/day, respectively, as potassium dichromate(VI) in the diet continuously for 9 weeks followed by an 8-week recovery period (NTP 1996b). Similarly, exposure of female F344/N rats and B6C3F1 mice to sodium dichromate dihydrate in drinking water at doses up to 20.9 and 27.9 mg chromium(VI)/kg/day, respectively, for 3 months or at doses up to 7.0 and 8.6 mg chromium(VI)/kg/day, respectively, for 2 years did not produce histopathological changes to the ovaries (NTP 2007, 2008a).

Several studies have reported increases in preimplantation losses and resorptions in rats and mice exposed to chromium(VI). However, for studies evaluating high concentration of chromium, it is possible that effects may, in part, be secondary to maternal toxicity. In addition, high concentration of chromium in food and water decrease palatability and can result in decreased food and drinking water consumption. Exposure of pregnant mice to 46 mg chromium(VI)/kg/day as potassium dichromate in drinking water during gestation resulted in increased preimplantation and postimplantation loss, and decreased litter size. Maternal body weight gain decreased at doses ≥98 mg chromium(VI)/kg/day (Trivedi et al. 1989). In female Swiss albino mice exposed for 20 days prior to mating to potassium dichromate in drinking water at concentrations that resulted in doses of 0, 52, 98, or 169 mg chromium(VI)/kg/day and then mated, the number of corpora lutea was decreased at 169 mg/kg/day, preimplantation loss and resorptions were increased at ≥98 mg/kg/day, and placental weights were decreased at ≥57 mg/kg/day (Junaid et al. 1996a). Increases in the number of resorptions were also found in female Swiss albino rats exposed to 37, 70, or 87 mg chromium(VI)/kg/day (as potassium dichromate in the drinking water) for 20 days prior to mating (Kanojia et al. 1996). Additional reproductive effects observed at 70 or 87 mg chromium(VI)/kg/day include decreased number of corpora lutea, decreased number of implantations, and increased number of preimplanation losses. A treatment-related increase in the length of estrus cycle was significantly different from controls only in the 87 mg chromium(VI)/kg/day group. Decreased mating, decreased fertility, and increased pre- and postimplantation loss were observed in female Druckrey rats receiving doses of 45, 89, and 124 mg chromium(VI)/kg/day (as potassium dichromate in the drinking water) for 3 months prior to mating; the 89 and 124 mg chromium(VI)/kg/day groups exhibited increased resorptions as well (Kanojia et al. 1998). A decrease in fertility (decreased number of implantations and viable fetuses) was observed in male and female Swiss mice that were exposed to 6 mg chromium(VI)/kg/day as potassium dichromate for 12 weeks and then were mated with unexposed males and females; however, the classification of non-viable fetuses was not presented in this report (Elbetieha and Al-

Hamood 1997). An increase in the number of mice with resorptions was also observed in the exposed females.

No reproductive effects were observed in a multigeneration reproductive assessment by continuous breeding study of BALB/c mice were fed a diet containing potassium dichromate(VI). Males and females were exposed to chromium for 7 days and then 20 pairs (F_0) in each dose group were allowed to continuously mate for 85 days (NTP 1997). The mean doses of chromium(VI) in F_0 animals were 6.8, 13.5, and 30.0 mg/kg/day. Litters produced during the 85-day mating period were examined at postnatal day 1. There were no treatment related changes in average litters/pair, number of live and dead pups per litter, sex ratios, pup weights, or changes in gestational time. There were no dose related gross pathological organ differences observed for both F_0 males and females, nor any differences in organ to body weight ratios. At the highest dose the F_0 females had lower mean body weights than control animals by about 7%. There were no effects on sperm number or motility, nor were there any increases in abnormal sperm morphology. Histopathological examination of livers and kidneys from F_0 males and females showed no changes that were treatment related. F_1 litters produced after 85 days were reared by the dam until weaning on postnatal day 21 then separated and allowed to mature for about 74 days. At that time, 20 pairs were allowed to mate and produce F_2 progeny. Mean exposures to chromium(VI) to F_1 animals were determined to be 7.8, 16.0, and 36.7 mg/kg/day. F_2 litters were reared by the dam until weaning on postnatal day 21 before being sacrificed. There were no differences in F_2 average litters/pair, number of live and dead pups per litter, sex ratios, pup weights, or changes in gestational time between exposed groups and controls. There were no dose-related gross pathological organ differences observed for both F_1 males and females, nor any differences in organ to body weight ratios. No histological lesions were observed in liver and kidney cells that were dose related, nor did chromium(VI) have any effects on estrous cycling.

Studies on the reproductive effects of chromium(III) yield conflicting results. Exposure to chromium(III) as chromium oxide did not cause reproductive effects in rats. Male and female rats fed 1,806 mg chromium(III)/kg/day as chromium oxide 5 days/week for 60 days before gestation and throughout the gestational period were observed to have normal fertility, gestational length, and litter size (Ivankovic and Preussmann 1975). A study by Bataineh et al. (1997) found significant alterations in sexual behavior (reductions in the number of mounts, increased postejaculatory interval, and decreased rates of ejaculation), aggressive behavior toward other males, and significantly lower absolute weight of testes, seminal vesicles, and preputial glands in male Sprague-Dawley rats exposed to 40 mg chromium(III)/ kg/day as chromium chloride in the drinking water for 12 weeks. Male fertility indices (assessed by

impregnation, number of implantations, and number of viable fetuses) did not appear to be adversely affected by exposure to chromium chloride, although the untreated females mated to treated males exhibited an increase in the total number of resorptions (Bataineh et al. 1997). In contrast, a decrease in the number of pregnant females was observed following the mating of unexposed females to male Swiss mice exposed to 13 mg chromium(III)/kg/day as chromium chloride (Elbetieha and Al-Hamood 1997). Impaired fertility (decreased number of implantations and viable fetuses) was also observed in females exposed to 5 mg chromium(III)/kg/day mated to unexposed males; however, no information on sperm count was reported and the definition and classification of viable fetuses were not provided (Elbetieha and Al-Hamood 1997). This study also found increased testes and ovarian weights and decreased preputial gland and uterine weights at 5 mg chromium(III)/kg/day. At lower concentrations of chromium chloride (9 mg chromium(III)/kg/day in the diet for 20 weeks), no alterations in testes or epididymis weights were observed in rats (Anderson et al. 1997b). A similar exposure to chromium(III) picolinate also did not result in testes or epididymis weight alterations (Anderson et al. 1997b). This study did not assess reproductive function. Mice exposed for 7 weeks to 9.1 mg chromium(III)/kg/day as chromium sulfate in the diet had reduced sperm count and degeneration of the outer cellular layer of the seminiferous tubules. Morphologically altered sperm occurred in BALB/c mice given diets providing 42.4 mg chromium(III)/kg/day as chromium sulfate (Zahid et al. 1990).

Exposure of rats and mice to high doses of chromium(III) compounds (chromium nicotinate and chromium picolinate) in the diet for 3 months or 2 years did not produce histopathological changes to male or female reproductive organs (NTP 2008b; Rhodes et al. 2005; Shara et al. 2005, 2007). In the 3-month studies on chromium picolinate, doses up to 505 and 506 mg chromium(III)/kg/day were evaluated in male and female F344/N rats, respectively, and doses up to 1,415 and 1,088 mg chromium(III)/kg/day were evaluated in male and female B6C3F1 mice, respectively; in the 2-year studies, doses up to 286 and 313 mg chromium(III)/kg/day were evaluated in male and female rats, respectively and doses up to 781 and 726 mg chromium(III)/kg/day, were evaluated in male and female mice, respectively (NTP 2008b; Rhodes et al. 2005). In addition, the 3-month study in rats and mice did not find any treatment-related effects on sperm count and motility or on estrous cycle (percentage of time spent in various estrous cycle stages or estrous cycle length, based on evaluation of vaginal cytology (NTP 2008b; Rhodes et al. 2005). Although the 3-month and 2-year studies on chromium nicotinate did not reveal any morphological changes to reproductive tissues of male and female Sprague-Dawley rats, only low doses were evaluated (up to 1.5 mg chromium(III)/kg/day for 3 months and up to 0.25 mg chromium(III)/kg/day for 2 years) (Shara et al. 2005, 2007).

As discussed in greater detail in Section 3.2.2.6, the reproductive system is also a target in the developing organism. Delayed vaginal opening and decreased relative weights of the uterus, ovaries, testis, seminal vesicle, and preputial glands were observed in mouse offspring exposed to potassium dichromate or chromium(III) chloride on gestational day 12 through lactation day 20 (Al-Hamood et al. 1998). Impaired fertility was observed in the chromium(III) chloride-exposed female offspring when they were mated with unexposed males (Al-Hamood et al. 1998); no effect on fertility was observed in the male offspring.

The highest NOAEL value and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3 for chromium(VI) and recorded in Table 3-4 and plotted in Figure 3-4 for chromium(III).

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to chromium or its compounds.

Several animal studies provide evidence that chromium(VI) is a developmental toxicant in rats and mice. A series of studies (Junaid et al. 1996a; Kanojia et al. 1996, 1998) were conducted to assess whether pre-mating exposure to potassium dichromate would result in developmental effects. In the first study, groups of 15 female Swiss albino mice were exposed to 0, 52, 98, or 169 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 20 days (Junaid et al. 1996a) and then mated with untreated males. At 52 mg chromium(VI)/kg/day, there was a 17.5% postimplantation loss over controls and a 30% decrease in fetal weight. At 98 mg/kg/day, there were decreases in the number of implantation sites, number of live fetuses, and fetal weight. There were also increases in the number of resorptions and number of pre- and postimplantation losses. At 169 mg chromium(VI)/kg/day, there was 100% preimplantation loss. The fetuses in the 98 mg/kg/day group had higher numbers of subdermal hemorrhagic patches and kinky short tails and decreased fetal body weight and crown rump length. Although there were no major skeletal abnormalities in any other treated animals, there was a significant reduction in ossification at 52 mg chromium(VI)/kg/day (53% compared to 12% for controls) and significant reduction in ossification in caudal, parietal and interparietal bones of fetuses at 98 mg chromium(VI)/kg/day. There were no significant soft tissue deformities in any of the treated fetuses. Although dosing occurred prior to mating, internal chromium levels remaining in females after mating may have been toxic to the conceptus that caused adverse developmental effects.

In the second study, female Swiss albino rats were exposed to potassium dichromate concentrations in the drinking water resulting in doses of 37, 70, or 87 mg chromium(VI)/kg/day for 20 days prior to mating (Kanojia et al. 1996). Lower gestational weight gain, increased postimplantation loss, and decreased number of live fetuses were observed in all treatment groups, relative to controls. Increased incidences of reduced fetal ossification in fetal caudal bones were reported at the 70 and 87 mg chromium(VI)/kg/day dose levels; additionally, the 87 mg chromium(VI)/kg/day dose group of fetuses exhibited increased incidences of reduced ossification in parietal and interparietal bones, as well as significant incidences of subdermal hemorrhagic thoracic and abdominal patches (42%), kinky tails (42%), and short tails (53%), relative to 0% in controls. No treatment-related gross visceral abnormalities were seen.

In the third study, groups of 10 female Druckrey rats were exposed to potassium dichromate in the drinking water for 3 months pre-mating at concentrations yielding dose levels of 45, 89, or 124 mg chromium(VI)/kg/day (Kanojia et al. 1998). Reduced maternal gestational weight gain, increased pre- and postimplantation loss, reduced fetal weight, fetal subdermal hemorrhagic thoracic and abdominal patches, increased chromium levels in maternal blood, placenta, and fetuses, and increased incidences of reduced ossification in fetal caudal bones were observed in all treatment groups. In addition, the 89 and 124 mg chromium(VI)/kg/day dose groups exhibited increased resorptions, reduced numbers of corpora lutea and fetuses per litter, reduced implantations, reduced placental weight, increased incidences of reduced ossification in fetal parietal and interparietal bones, and reduced fetal crown-rump length. No treatment-related gross visceral abnormalities were seen. A decreased number of pregnancies were observed in mated female rats administered 35.7 mg chromium(VI)/mg/day as potassium dichromate by gavage on gestational days 1–3; exposure on gestational days 4–6 decreased the number of viable fetuses and increased the number of resorptions, but did not alter the number of pregnancies (Bataineh et al. 2007).

Exposure of pregnant mice to 57 mg chromium(VI)/kg/day as potassium dichromate in drinking water during gestation resulted in embryo lethal effects (i.e., increased resorptions and increased post-implantation loss), gross abnormalities (i.e., subdermal hemorrhage, decreased cranial ossification, tail kinking), decreased crown-rump length, and decreased fetal weight. The incidence and severity of abnormalities increased at higher doses. Maternal toxicity, evidenced by decreased body weight gain, occurred at doses ≥120 mg chromium(VI)/kg/day. No implantations were observed in the dams given 234 mg chromium(VI)/kg/day (Trivedi et al. 1989).

Groups of 10 female Swiss albino mice received chromium(VI) as potassium dichromate in drinking water during organogenesis on days 6–14 at levels that provided 0, 53.2, 101.1, and 152.4 mg chromium(VI)/kg/day (Junaid et al. 1996b). No notable changes in behavior or clinical signs were observed in control or treated animals. Reduction of gestational weight gains of 8.2 and 30% were observed for the animals in the intermediate- and high-dose groups. The number of dead fetuses was higher in the high-dose group and fetal weight was lower in both intermediate- and high-dose groups (high dose=1.06 g, intermediate dose=1.14 g) as compared to the control value of 1.3 g. The number of resorption sites was 0.31 for controls, 1.00 for the low dose, 1.70 for the intermediate dose, and 2.30 for the high dose, demonstrating a dose-response relationship. The studies also showed that there was a significantly greater incidence of postimplantation loss in the two highest-dose groups of 21 and 34.60% as compared to control value of 4.32%. No significant gross structural abnormalities in any of the treated dosed groups were observed except for drooping of the wrist (carpal flexure) and subdermal hemorrhagic patches on the thoracic and abdominal regions in 16% in the offspring of the high-dose group. Significant reduced ossification in nasal frontal, parietal, interparietal, caudal, and tarsal bones were observed only in the 152.4 mg chromium(VI)/kg/day-treated animals.

Impaired development of the reproductive system was observed in the offspring of female BALB/c mice exposed to 66 mg chromium(VI)/kg/day as potassium chromate in the drinking water on gestation day 12 through lactation day 20 (Al-Hamood et al. 1998). A significant delay in vaginal opening was observed. Significant decreases in the numbers of pregnant animals, of implantations, and of viable fetuses were also observed when the female offspring were mated at age 60 days with unexposed males. No developmental effects were observed in the male offspring. In pregnant rats exposed to 8 mg chromium(VI)/kg/day as potassium chromate in drinking water on gestational days 6 through 15, pre- and postimplanation losses and the number of resorbed and dead fetuses per litter were increased compared to controls (Elsaieed and Nada 2002). Fetal weight was significantly decreased by 67% and the number of visceral (renal pelvis dilatation) and skeletal (incomplete ossification of skull bone) anomalies per litter were significantly increased. No effects on fetal body weight or the number of fetuses per litter were observed in mice exposed to 4.8 mg chromium(VI)/kg/day as sodium dichromium dihydrate or 2.4 mg chromium(VI)/kg/day as potassium dichromate in drinking water on gestational days 0 through 18; however, no additional assessments on fetal development were conducted in this study (De Flora et al. 2006).

Three studies examined the developmental toxicity of chromium(III) following oral maternal exposure. In the first study, no developmental effects were observed in offspring of rats fed 1,806 mg

chromium(III)/kg/day as chromium oxide 5 days/week for 60 days before mating and throughout gestation (Ivankovic and Preussmann 1975). In contrast, reproductive effects have been observed in the offspring of mice exposed to chromium(III) chloride. Significant decreases in the relative weights of reproductive tissues (testes, seminal vesicles, and preputial glands in males; ovaries and uterus in females) were observed in the offspring of BALB/c mice exposed to 74 mg chromium(III)/kg/day as chromium(III) chloride in the drinking water on gestation day 12 through lactation day 20 (Al-Hamood et al. 1998). A significant delay in timing of vaginal opening was also noted in the female offspring. At age 60 days, the male and female offspring were mated with unexposed animals. No significant alterations in fertility (number of pregnant animals, number of implantations, number of viable fetuses, and total number of resorptions) were observed in the exposed males. A significant decrease in the number of pregnant females (62.5 versus 100% in controls) was observed among the female offspring mated with untreated males. The conflicting results between the Ivankovic and Preussmann (1975) study and the Al-Hamood et al. (1998) study may be a reflection on the developmental end points examined or the differences in the species tested. In rats administered 33.6 mg chromium(III)/kg/day (only dose tested) by gavage as chromium chloride on gestational days 1–3, a decreased number of pregnancies were observed; however, when exposed on gestational days 4–6, no effects on pregnancy rates, implantations, viable fetuses, or resorptions were observed (Bataineh et al. 2007).

The NOAEL and LOAEL values for developmental effects in each species are recorded in Table 3-3 and plotted in Figure 3-3 for chromium(VI) and recorded in Table 3-4 and plotted in Figure 3-4 for chromium(III).

3.2.2.7 Cancer

Studies of associations between environmental exposures to chromium and cancer outcomes in humans are limited to several ecological studies (Beaumont et al. 2008; Fryzek et al. 2001; Zhang and Li 1987). These types of studies investigate possible associations between rates of selected diseases (e.g., cancer deaths) within a geographic population and some measure of average exposure to chromium (e.g., drinking water chromium concentrations or location with respect to potential sources of exposure). Actual exposures to individuals are not determined and therefore, exposure misclassification bias often contributes to uncertainty regarding associations between outcomes and exposure. Findings from ecological studies are mixed and do not strongly support associations between cancer mortality and exposures to chromium. One study did find significantly higher stomach cancer death rates in areas where well water chromium levels had been elevated (Beaumont et al. 2008).

An ecological study of an area near a ferrochromium production plant in the Liaoning Province, China compared cancer mortality in locations that had relatively high or low chromium concentrations in well water (Beaumont et al. 2008; Zhang and Li 1987). The most recent study of the area estimated cancer mortality rates (cancers deaths per person-year in an 8-year observation period) based on mortality records for the period 1970–1978 (Beaumont et al. 2008). The province was divided into nine areas, four of which were designated as no (or low) chromium (groundwater concentration <0.001 mg Cr/L) and five which were designated as high chromium. The main sources of chromium in well water were from discharges from the plant to surface water and groundwater, which began operating in 1961. Chromium levels in well water from samples collected in the contaminated areas in 1965 (by this time, full-scale production was occurring) ranged from 0.6 to 20 mg/L with 15% of wells having concentrations >2 mg/L. Total number of cancer deaths were 80 (of 98,458 person-years) in the high chromium areas and 182 (of 252,277 person-years) in the comparison areas. Age-adjusted cancer mortality rate ratios (rate in high regions/rate in low regions) were 1.82 (95% CI 1.11–2.91) for stomach cancer, 1.15 (95% CI 0.62–2.05) for lung cancer, 0.86 (95% CI 0.53–1.36) for other cancers, and 1.13 (95% CI 0.86–1.46) for all cancer.

An ecological 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 additives in cooling tower water at the gas plants during the period 1950 to approximately 1980. Mortality records for zip codes for the cities of Kettleman City (in Kings County), and Hinkely 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.

An ecological study compared levels of chromium (and other chemicals) in drinking water in 453 Nebraska communities with death rates in these areas (Bednar and Kies 1991). Data on chromium in drinking water were obtained for the year period 1986–1987, and mortality data was obtained for the year 1986. Mean chromium concentration in drinking water was 0.002 mg/L (range <0.001–0.01). Linear correlation (Pearson) between chromium levels and death from chronic lung disease was -0.101 (p=0.03).

Chronic exposure to chromium(VI) as sodium dichromate in drinking water resulted in increased incidence of neoplasms of the digestive tract in mice and rats (NTP 2008a). Groups of 50 male and 50 female F344/N rats were exposed to drinking water containing 0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate for 2 years. NTP (2008a) calculated 2-year mean daily doses of 0, 0.6, 2.2, 6, or 17 mg sodium dichromate dihydrate/kg/day (equivalent to 0, 0.21, 0.77, 2.1 or 5.9 mg chromium(VI)/kg/day) in male rats and, 0, 0.7, 2.7, 7, or 20 mg sodium dichromate dihydrate/kg/day (equivalent to 0, 0.24, 0.94, 2.4, and 7.0 mg chromium(VI)/kg/day) in female rats. Incidences of squamous epithelial neoplasms of the oral mucosa and tongue were elevated in rats exposed to sodium dichromate compared to controls, with significant increased mortality-adjusted incidence in males at the 5.9 mg chromium(VI)/kg/day dose (15.7 versus 0% in controls, $p=0.007$), and in females at the 7.0 mg chromium(VI)/kg/day (23.9 versus 2.2% in controls, $p<0.001$). In both male and female rats, there was a significant dose trend for digestive tract neoplasms ($p<0.001$). Groups of 50 male B6C3F1 mice were exposed to 0, 14.3, 28.6, 85.7, or 257.4 mg sodium dichromate dihydrate/L, and 50 female B6C3F1 mice were exposed to drinking water concentrations of 0, 14.3, 57.3, 172, or 516 mg sodium dichromate dihydrate/L. NTP (2008a) calculated 2-year mean daily doses of sodium dichromate dihydrate in male mice of 1.1, 2.6, 7 or 17 mg/kg/day (equivalent to 0, 0.38, 0.91, 2.4 and 5.9 mg chromium(VI)/kg/day); and in female mice of 0, 1.1, 3.9, 9, or 25 mg/kg/day (equivalent to 0, 0.38, 1.4, 3.1, or 8.7 mg chromium(VI)/kg/day). Incidences of neoplasms of the of the small intestine (duodenum, jejunum, or ileum) were elevated in mice exposed to sodium dichromate compared to controls, with significant increased mortality-adjusted incidence in males at the 2.4 (15.1 versus 2.2% in controls, $p=0.032$) or 5.9 mg chromium(VI)/kg/day dose (43.8 versus 2.2% in controls, $p=0.001$), and in females at the 3.1 (36.3 versus 2,2% in controls) or 8.7 mg chromium(VI)/kg/day (45.9 versus 2.2% in controls, $p<0.001$). In both male and female mice, there was a significant dose trend for digestive tract neoplasms ($p<0.001$). NTP (2008a) concluded that the results of these studies provided 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 in male and female B6C3F1 mice based on increased incidences of neoplasms of the small intestine (duodenum, jejunum, or ileum).

The carcinogenicity of chromium(VI) was evaluated in mice exposed potassium chromate in drinking water at 9 mg chromium(VI)/kg/day for three generations (880 days) (Borneff et al. 1968). In treated mice, 2 of 66 females developed forestomach carcinoma and 9 of 66 females and 1 of 35 males developed forestomach papilloma. The vehicle controls also developed forestomach papilloma (2 of 79 females, 3 of 47 males) but no carcinoma. The incidence of forestomach tumors in the treated mice was not significantly higher than controls. Although study authors concluded that evidence of carcinogenicity

was equivocal, statistical analysis of these data (performed by Syracuse Research Corporation) using Fischer's exact test shows statistically significant increases in the incidence of adenoma or carcinomas (forestomach) (p=0.0067) and in the incidence of adenomas (forestomach) alone (p=0.027), compared to control. In this same study, coexposure to both potassium chromate and 3,4-benzpyrene in a similar protocol showed that potassium chromate did not potentiate the carcinogenicity of 3,4-benzpyrene (Borneff et al. 1968). Exposure of female hairless mice to ultraviolet light in combination with chromium(VI) as potassium chromate in drinking water at concentrations of 2.5 or 5.0 mg potassium chromate(VI)/L (approximately 0.18, or 0.35 mg chromium(VI)/kg/day) for 182 days, or in the diet at concentrations of 0, 2.5, or 5.0 mg potassium chromate(VI)/kg food (approximately 0.13, or 0.26 mg chromium(VI)/kg/day) for 26 weeks, produced an increased incidence of skin tumors compared to animals exposed to UV light alone or chromium(VI) alone (Davidson et al. 2004; Uddin et al. 2007). Exposure to chromium(VI) alone did not result in neoplasms.

Chronic exposure to chromium(III) as chromium picolinate dihydrate in the diet resulted in increased incidence of neoplasms of the preputial gland in male rats; however, no increased neoplasms were observed in female rats, or in male or female mice (NTP 2008b). Groups of 50 male and 50 female F344/N rats were fed a diet containing 0, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate for 2 years. NTP (2008b) calculated 2-year mean daily doses of chromium picolinate monohydrate of 0, 90, 460, and 2,400 mg/kg/day (equivalent to 0, 11, 55, or 286 mg chromium(III)/kg/day) in male rats and 0, 100, 510, and 2,630 mg/kg/day (equivalent to 0, 12, 61, or 313 mg chromium(III)/kg/day) in female rats. Mortality-adjusted incidence of adenoma of the preputial gland of male rats was significantly elevated in rats that received 55 mg chromium(III)/kg/day (14.9 versus 2.2% in controls, p=0.031), but not in rats exposed to lower dose or the higher dose (286 mg chromium(III)/kg/day), and there was no significant dose trend for the neoplasm. Incidences of neoplasms were not significantly different from controls in females, including neoplasms of the clitoral gland. Groups of 50 male and 50 female F6C3F1 mice were fed a diet containing 0, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate for 2 years. NTP (2008b) calculated 2-year mean daily doses of chromium picolinate monohydrate of 0, 250, 1,200, and 6,565 mg/kg/day (equivalent to 0, 30, 143, 2.1, or 781 mg chromium(III)/kg/day) in male mice and 100, 510, and 2,630 mg/kg/day (equivalent to 0, 29, 143, or 726 mg chromium(III)/kg/day) in female mice. No neoplasms or lesions were attributed to exposure to chromium picolinate monohydrate in male or female mice. NTP (2008b) concluded that evidence for carcinogenicity of chromium picolinate in male rats was equivocal and that the study provided no evidence of carcinogencity in mice.

No evidence of carcinogenicity was observed in male or female rats fed diets containing chromium oxide at 2,040 mg chromium(III)/kg/day 5 days/week for 2 years. Moreover, no evidence of carcinogenicity was found in the offspring of these rats after 600 days of observation (Ivankovic and Preussmann 1975).

The Cancer Effect Levels (CELs) for chromium(VI) are recorded in Table 3-3 and plotted in Figure 3-3.

3.2.3 Dermal Exposure

Some chromium(VI) compounds, such as chromium trioxide (chromic acid), potassium dichromate, potassium chromate, sodium dichromate, and sodium chromate, are very caustic and can cause burns upon dermal contact. These burns can facilitate the absorption of the compound and lead to systemic toxicity.

3.2.3.1 Death

A 49-year-old man with an inoperable carcinoma of the face was treated with chromic acid crystals. Severe nephritis occurred following the treatment with the chromium(VI) compounds. Death occurred 4 weeks after exposure (Major 1922). Twelve individuals died as a result of infection to necrotic areas of the skin that were caused by application of a salve made up with potassium chromate used to treat scabies. Renal failure was observed. Autopsies revealed fatty degeneration of the heart, hyperemia and necrosis of kidney tubules, and hyperemia of the gastric mucosa (Brieger 1920).

Single-dose dermal LD_{50} values in New Zealand rabbits exposed to chromium(VI) as sodium chromate, sodium dichromate, potassium dichromate, and ammonium dichromate were determined by Gad et al. (1986). LD_{50} values ranged from 361 to 553 mg chromium(VI)/kg for females and from 336 to 763 mg chromium(VI)/kg for males. Signs of toxicity included dermal necrosis, eschar formation, dermal edema and erythema, diarrhea, and hypoactivity. The dermal LD_{50} value for chromium trioxide was 30 mg chromium(VI)/kg for combined sexes (American Chrome and Chemicals 1989). In male and female Sprague-Dawley rats, no mortalities were observed following a single dermal application of 621.6 mg chromium(III)/kg as chromium nicotinate (Shara et al. 2005).

The LD_{50} values are recorded in Table 3-5 for chromium(VI) and Table 3-6 for chromium(III).

Table 3-5 Levels of Significant Exposure to Chromium VI - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Death								
Rabbit (Fischer- 344)	24 hr					30 mg/kg (LD50)	American Chrome and Chemicals 1989 CrO3 (VI)	
Rabbit (New Zealand)	once					763 M mg/kg (LD50)	Gad et al. 1986 (NH4)2Cr2O7 (VI)	
						549 F mg/kg (LD50)		
Rabbit (New Zealand)	once					403 M mg/kg (LD50)	Gad et al. 1986 K2Cr2O7 (VI)	
						490 F mg/kg (LD50)		
Rabbit (New Zealand)	once					336 M mg/kg (LD50)	Gad et al. 1986 Na2Cr2O72H2O (VI)	
						361 F mg/kg (LD50)		
Rabbit (New Zealand)	2 d					426 M mg/kg (LD50)	Gad et al. 1986 Na2CrO4 (VI)	
						553 F mg/kg (LD50)		

Table 3-5 Levels of Significant Exposure to Chromium VI - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	LOAEL NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Systemic							
Rat (NS)	once	Hepatic		0.175 Percent (%) (altered carbohydrate metabolism)		Merkur'eva et al. 1982 K2Cr2O7 (VI)	
		Dermal		0.175 Percent (%) (dermatitis)			
Gn Pig (albino)	once	Dermal			1.9 M mg/kg (skin corrosion)	Samitz 1970 K2Cr2O7 (VI)	
Gn Pig (NS)	3 d 1 x/d	Dermal		0.35 mg/kg (skin ulcers)		Samitz and Epstein 1962 K2Cr2O7 (VI)	
Rabbit (NS)	5 min or 24 hr	Ocular	0.1 M ml			Fujii et al. 1976 Na2CrO4 and Na2Cr2O7 (VI)	
Rabbit (New Zealand)	4 hr	Dermal		55 mg/kg (necrosis, erythema, edema)		Gad et al. 1986 (NH4)2Cr2O7 (VI)	
Rabbit (New Zealand)	4 hr	Dermal		47 M mg/kg (erythema, edema, necrosis)		Gad et al. 1986 K2Cr2O7 (VI)	
Rabbit (New Zealand)	4 hr	Dermal		47 M mg/kg (necrosis, erythema, edema)		Gad et al. 1986 Na2Cr2O7 (VI)	

Table 3-5 Levels of Significant Exposure to Chromium VI - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Rabbit (New Zealand)	4 hr	Dermal		42 M mg/kg (erythema, edema)		Gad et al. 1986 Na2CrO4 (VI)	
Immuno/ Lymphoret							
Human	once			0.175 Percent (%) (positive patch test)		Engebrigsten 1952 K2Cr2O7 (VI)	
Human	48 hr			0.001 Percent (%) (increased skin thicknes and blood flow)		Eun and Marks 1990 K2Cr2O7 (VI)	
Human	48 hr (NS)			1 B mg/L (positive patch test)		Hansen et al. 2003 K2Cr2O7 (VI)	
Human	48 hr			0.18 Percent (%) (positive patch test)		Hansen et al. 2006b K2Cr2O7 (VI)	
Human	48 hr			0.26 M Percent (%) (erythema)		Levin et al. 1959 CrO3 (VI)	
Human	once		0.0013 µg/mm2	0.0026 µg/mm2 (positive patch test)		Mali et al. 1966 K2Cr2O7 (VI)	
Human	once			0.018 µg/cm² (positive patch test)		Nethercott et al. 1994 K2Cr2O7 (VI)	

Table 3-5 Levels of Significant Exposure to Chromium VI - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Human	2 d			0.175 Percent (%) (positive patch test)		Newhouse 1963 K2Cr2O7 (VI)	
Human	48 hr			0.175 Percent (%) (chromium allergy)		Peltonen and Fraki 1983 K2Cr2O7 (VI)	
Human	once			0.09 mg (erythema)		Samitz and Shrager 1966 K2Cr2O7 (VI)	
Human	once			0.09 Percent (%) (positive patch test)		Wahba and Cohen 1979 K2Cr2O7 (VI)	
Human	once			0.09 Percent (%) (positive patch test)		Winston and Walsh 1951 Na2Cr2O7 (VI)	
Gn Pig (albino)	once			0.009 mg/kg (contact sensitivity)		Gross et al. 1968 K2Cr2O7 (VI)	
Gn Pig (NS)	once			0.04 F mg/kg (erythematic reaction)		Jansen and Berrens 1968 K2Cr2O7 (VI)	

Table 3-5 Levels of Significant Exposure to Chromium VI - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE							
Immuno/ Lymphoret							
Mouse (BALB/c or ICR)	18 d			0.35 Percent (%) (contact sensitivity)		Mor et al. 1988 K2Cr2O7 (VI)	
CHRONIC EXPOSURE							
Systemic							
Human	>1 yr (occup)	Dermal		0.03 M mg/m³ (ulcerated skin)		Gibb et al. 2000a CrO3 (VI)	
				0.029 M mg/m³ (dermatitis)			
				0.027 M mg/m³ (burn)			
				0.025 M mg/m³ (irritated skin)			
Human	7.5 yr avg (range 3-16 yr) (occup)	Resp		0.004 M mg/m³ (nasal septum ulceratior and perforation)		Lucas and Kramkowski 1975 CrO3 (VI)	
		Gastro		0.004 M mg/m³ (possible gastritis, ulcers			
		Dermal		0.005 M mg/m³ (chrome holes)			

avg = average; d = day(s); F = female; Gastro = gastrointestinal; Gn Pig = guinea pig; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; x = times; yr = year(s)

Table 3-6 Levels of Significant Exposure to Chromium III - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Systemic							
Gn Pig (NS)	3 d 1 x/d	Dermal	1 mg/kg			Samitz and Epstein 1962 Cr2(SO4)3 (III)	
Immuno/ Lymphoret							
Human	48 hr			0.37 Percent (%) (positive patch test)		Fregert and Rorsman 1964 CrCl3.6H2O (III)	
Human	48 hr (NS)			6 mg/L (positive patch test)		Hansen et al. 2003 CrCl3.6H2O (III)	
Human	48 hr			3.7 Percent (%) (positive patch test)		Hansen et al. 2006b CrCl3 (III)	
Human	once			0.16 µg/mm2 (positive patch test)		Mali et al. 1966 CrCl3 (III)	
Human	once		33 µg/cm²			Nethercott et al. 1994 CrCl3 (III)	
Human	once			0.33 mg (erythema)		Samitz and Shrager 1966 Cr2(SO4)3 (III)	
Human	once			0.08 mg (erythema)		Samitz and Shrager 1966 CrCl3 (III)	

Table 3-6 Levels of Significant Exposure to Chromium III - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Gn Pig (albino)	once			0.004 mg/kg (erythematic reaction)		Gross et al. 1968 CrCl3 (III)	
Gn Pig (NS)	once			0.03 F mg/kg (erythematic reaction)		Jansen and Berrens 1968 Cr2(SO4)3 (III)	

d = day(s); F = female; Gn Pig = guinea pig; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; x = times

3.2.3.2 Systemic Effects

Several reports of health effects in individuals treated with potassium dichromate are discussed below (Brieger 1920; Major 1922; Smith 1931). The results of these studies should be interpreted cautiously because pre-existing conditions may have contributed to the observed effects. The highest NOAEL value and all reliable LOAEL values for dermal effects in each species and duration category are recorded in Table 3-5 for chromium(VI) and Table 3-6 for chromium(III).

Respiratory Effects. Occupational exposure to chromium compounds results in direct contact of mucocutaneous tissue, such as nasal and pharyngeal epithelium, due to inhalation of airborne dust and mists of these compounds. Such exposures have led to nose and throat irritation and nasal septum perforation. Because exposure is to airborne chromium, studies noting these effects are described in Section 3.2.1.2.

A case report of a man who was admitted to a hospital with skin ulcers on both hands due to dermal exposure to ammonium dichromate in a planographic printing establishment where he had worked for a few months noted that he also had breathing difficulties. However, because he also had many previous attacks of hay fever and asthma, it was not possible to distinguish whether his breathing difficulties were caused by or exacerbated by dermal exposure to ammonium dichromate (Smith 1931).

No studies were located regarding respiratory effects in animals after dermal exposure to chromium or its compounds.

Cardiovascular Effects. Information regarding cardiovascular effects in humans after dermal exposure to chromium or its compounds is limited. Weak, thready, and markedly dicrotic pulse developed ≈1.5 hours after a salve made up with potassium chromate to treat scabies was applied to skin of an unspecified number of individuals. Some of the people died as a result of infection to the exposed area, and autopsy revealed degeneration of the heart (Brieger 1920).

No studies were located regarding cardiovascular effects in animals after dermal exposure to chromium or its compounds.

Gastrointestinal Effects. Vomiting occurred soon after application of a salve made up of potassium chromate to the skin of an unspecified number of individuals for the treatment of scabies. Some of these

individuals died as a result of infection of the exposed area, and autopsy revealed hyperemia of the gastric mucosa (Brieger 1920).

Diarrhea was reported in New Zealand rabbits exposed to lethal concentrations of chromium(VI) compounds (Gad et al. 1986).

Hematological Effects. Severe leukocytosis, with notable increases in immature polymorphonuclear cells, myelocytes, and myeloblasts and nucleated red cells and Howell-Jolly bodies, indicative of hemolytic anemia were observed in individuals after application of a salve that contained potassium chromate to treat scabies (Brieger 1920). Leukocytosis was also described in a case report of a man who was admitted to a hospital with skin ulcers on both hands due to dermal exposure to ammonium dichromate in a planographic printing establishment, where he had worked for a few months (Smith 1931). It should be noted that the man had a history of asthma.

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

Musculoskeletal Effects. Information regarding musculoskeletal effects in humans after dermal exposure to chromium or its compounds is limited to a case report. A man was admitted to a hospital with skin ulcers on both hands due to dermal exposure to ammonium dichromate in a planographic printing establishment, where he had worked for a few months. He also had tenderness and edema of the muscles of the extremities (Smith 1931).

No studies were located regarding musculoskeletal effects in animals after dermal exposure to chromium or its compounds.

Hepatic Effects. No reliable studies were located regarding hepatic effects in humans after dermal exposure to chromium compounds.

Information regarding liver effects in animals after dermal exposure to chromium or its compounds is limited. A single application of 0.5% potassium dichromate (0.175% chromium(VI)) to the shaved skin of rats resulted in increased levels of serotonin in the liver, decreased activities of acetylcholinesterase and cholinesterase in the plasma and erythrocytes, increased levels of acetylcholine in the blood, and increased glycoprotein hexose in the serum. These effects may indicate alterations in carbohydrate

metabolism (Merkur'eva et al. 1982). Application of 50 mg chromium/kg/day (specific chemical or valence state not reported) for 30 days to clipped skin under occluded conditions to female guinea pigs produced small increases in enzyme activities in liver tissue, specifically asparatate aminotransferase (17%), alanine aminotransferase (2%), acid phosphatase (16%), and gamma glutamyl transpeptidase (54%), compared to untreated controls (Mathur 2005). Microscopic evaluation of the liver showed "shrunken" hepatocytes and thickening of the walls of hepatic arteries.

Renal Effects. Acute nephritis with albuminuria and oliguria, polyuria, and nitrogen retention were observed in individuals after application of a salve that contained potassium chromate. These effects disappeared in individuals who survived. Autopsy of people who died revealed hyperemia and tubular necrosis (Brieger 1920). Acute nephritis with polyuria and proteinuria were also described in a man who was admitted to a hospital with skin ulcers on both hands due to dermal exposure to ammonium dichromate in a planographic printing establishment where he had worked for a few months (Smith 1931). A 49-year-old man with an inoperable carcinoma of the face was treated with chromic acid crystals. Severe nephritis occurred after treatment with the chromium(VI) compound. Urinalysis revealed marked protein in the urine. Death resulted 4 weeks after exposure. A postmortem examination of the kidneys revealed extensive destruction of the tubular epithelium (Major 1922).

Application of 50 mg chromium/kg/day (specific chemical or valence state not reported) for 30 days to clipped skin under occluded conditions to female guinea pigs produced increases in enzyme activities in renal tissue, specifically asparatate aminotransferase (8%), alanine aminotransferase (96%), and acid phosphatase (4%), compared to untreated controls (Mathur 2005). Microscopic evaluation of the kidney showed lobularization of the glomerular tuft and congestion of capillaries. No additional information on renal effects of dermal exposure to chromium(VI) or chromium(III) compounds was identified.

Dermal Effects. Occupational exposure to airborne chromium compounds has been associated with effects on the nasal septum, such as ulceration and perforation. These studies are discussed in Section 3.2.1.2 on Respiratory Effects. Dermal exposure to chromium compounds can cause contact allergic dermatitis in sensitive individuals, which is discussed in Section 3.2.3.3. Skin burns, blisters, and skin ulcers, also known as chrome holes or chrome sores, are more likely associated with direct dermal contact with solutions of chromium compounds, but exposure of the skin to airborne fumes and mists of chromium compounds may contribute to these effects.

Acute dermal exposure of humans to chromium(VI) compounds causes skin burns. Necrosis and sloughing of the skin occurred in individuals at the site of application of a salve containing potassium chromate. Twelve of 31 people died as a result of infection of these areas (Brieger 1920). In another case, a man who slipped at work and plunged his arm into a vat of chromic acid had extensive burns and necrosis on his arm (Cason 1959).

Longer-term occupational exposure to chromium compounds in most chromium-related industries can cause deep penetrating holes or ulcers on the skin. A man who had worked for a few months in a planographic printing establishment, where he handled and washed sheets of zinc that had been treated with a solution of ammonium dichromate, had skin ulceration on both hands (Smith 1931).

In an extensive survey to determine the health status of chromate workers in seven U.S. chromate production plants, 50% of the chromate workers had skin ulcers or scars. In addition, inflammation of oral structures, keratosis of the lips, gingiva, and palate, gingivitis, and periodontis due to exposure of these mucocutaneous tissues to airborne chromium were observed in higher incidence in the chromate workers than in controls. Various manufacturing processes in the plants resulted in exposure of workers to chromite ore (mean time-weighted concentration of 0–0.89 mg chromium(III)/m^3 air); water-soluble chromium(VI) compounds (0.005–0.17 mg chromium(VI)/m^3); and acid-soluble/water-insoluble chromium compounds (including basic chromium sulfate), which may or may not entirely represent chromium(III) (0–0.47 mg chromium/m^3 air) (PHS 1953). Among 258 electroplating workers exposed to chromium trioxide fumes at 0.1 mg chromium(VI)/m^3 for <1 year, 5% developed dental lesions, consisting of yellowing and wearing down of the teeth (Gomes 1972).

Chronic exposure of chrome chemical production workers produced dermal symptoms, including irritated and ulcerated skin, dermatitis, and burns (Gibb et al. 2000a). Medical records of 2,307 male workers (all nonsmokers) employed at a chromate production plant in Baltimore, Maryland between 1950 and 1974 were evaluated to determine the percentage of workers reporting clinical symptoms, mean time of employment to first diagnosis of symptoms, and mean exposure to chromium(VI) at the time of first diagnosis (exposure for each worker was the annual mean in the area of employment during the year of first diagnosis). Ulcerated skin occurred in 31.6% of workers, at a mean exposure of 0.029 mg Cr(VI)/m^3 and a mean time to first diagnosis of 373 days. Ulcerated skin was significantly associated with chromium(VI) exposure ($p=0.004$), with a relative risk of 1.11. Burns were observed in 31.4% of workers, with a mean exposure and time to onset of 0.027 mg/m^3 and 409 days, respectively. Dermatitis was observed in 18.5% of workers, with a mean exposure and time to onset of 0.029 mg/m^3 and 624 days,

respectively. Irritated skin was observed in 15.1% of workers, with a mean exposure and time to onset of 0.025 mg/m^3 and 719 days, respectively.

Irritation and ulceration of the buccal cavity, as well as chrome holes on the skin, were also observed in workers in a chrome plating plant where poor exhaust resulted in excessively high concentrations of chromium trioxide fumes (Lieberman 1941). Electroplaters in Czechoslovakia exposed to an average of 0.414 mg chromium(VI)/m^3 above the plating baths also had high incidences of buccal cavity changes, including chronic tonsillitis, pharyngitis, and papilloma (Hanslian et al. 1967). In a study of 303 electroplating workers in Brazil, whose jobs involve working with cold chromium trioxide solutions, >50% had ulcerous scars on the hands, arms, and feet. Air monitoring revealed that most workers were exposed to ≥0.1 mg chromium(VI)/m^3, but even those exposed to <0.1 mg chromium(VI)/m^3 developed lesions (Gomes 1972). Chrome holes were also noted at high incidence in chrome platers in Singapore, while controls had no skin ulcers (Lee and Goh 1988). The incidence of skin ulcers was significantly increased in a group of 997 chrome platers compared with 1,117 controls. The workers had been exposed to chromium(VI) in air and in dust. The air levels were generally <0.3 mg chromium(VI)/m^3, and dust levels were generally between 0.3 and 97 mg chromium(VI)/g (Royle 1975b). In a NIOSH Health Hazard Evaluation of an electroplating facility in the United States, seven workers reported past history of skin sores, and nine had scars characteristic of healed chrome sores. The workers had been employed for an average of 7.5 years and were exposed to a mean concentration of 0.004 mg chromium(VI)/m^3 in air. In addition, spot tests showed widespread contamination of almost all workroom surfaces and hands (Lucas and Kramkowski 1975).

An early report of cases of chrome ulcers in leather tanners noted that the only workmen in tanneries who suffered chrome holes were those who handled dichromate salts. In one of these cases, the penetration extended into the joint, requiring amputation of the finger (Da Costa et al. 1916). In a medical survey of a chemical plant that processed chromite ore, 198 of 285 workers had chrome ulcers or scars on the hands and arms. These workers had been exposed to one or more chromium(VI) compounds in the form of chromium trioxide, potassium dichromate, sodium dichromate, potassium chromate, sodium chromate, and ammonium dichromate (Edmundson 1951).

Similar dermal effects have been observed in animals. Dermal application of chromium(VI) compounds to the clipped, nonabraded skin of rabbits at 42–55 mg/kg resulted in skin inflammation, edema, and necrosis. Skin corrosion and eschar formation occurred at lethal doses (see Section 3.2.3.1) (Gad et al. 1986). Application of 0.01 or 0.05 mL of 0.34 molar solution of potassium dichromate (0.35 mg

chromium(VI) or 1.9 mg chromium(VI)/kg) to the abraded skin of guinea pigs resulted in skin ulcers (Samitz 1970; Samitz and Epstein 1962). Similar application of 0.01 mL of a 1 molar solution of chromium sulfate (1 mg chromium(III)/kg) however, did not cause skin ulcers in guinea pigs (Samitz and Epstein 1962). In a primary dermal irritation test, application of 88 mg chromium(III) as chromium nicotinate in corn oil to clipped skin of male and female New Zealand albino rabbits produced very slight erythema after 1 hour after application, with no signs of dermal irritation 48 hours after application (Shara et al. 2005).

Dermal sensitization due to hypersensitivity to chromium is discussed in Section 3.2.3.3.

Ocular Effects. Medical records of 2,307 male workers (all nonsmokers) employed at a chromate production plant in Baltimore, Maryland between 1950 and 1974 were evaluated to determine the percentage of workers reporting clinical symptoms, mean time of employment to first diagnosis of symptoms, and mean exposure to chromium(VI) at the time of first diagnosis (exposure for each worker was the annual mean in the area of employment during the year of first diagnosis) (Gibb et al. 2000a). Conjunctivitis was reported on 20.0% of the study population, at a mean exposure level of 0.025 mg Cr(VI)/m^3 and a mean time-to-onset of 604 days.

Direct contact of the eyes with chromium compounds also causes ocular effects. Corneal vesication was described in a worker who accidentally got a crystal of potassium dichromate or a drop of a potassium dichromate solution in his eye (Thomson 1903). In an extensive study of chromate workers in seven U.S. chromate production plants, eyes were examined because accidental splashes of chromium compounds into the eye had been observed in these plants. Congestion of the conjunctiva was found in 38.7% of the 897 workers, discharge in 3.2%, corneal scaring in 2.3%, any abnormal finding in 40.8%, and burning in 17.0%, compared with respective frequencies of 25.8, 1.3, 2.6, 29.0, and 22.6% in 155 nonchromate workers. Only the incidences of congestion of the conjunctiva and any abnormal findings were significantly higher in the exposed workers than in the controls (PHS 1953).

Instillation of 0.1 mL of a 1,000 mg chromium(VI)/L solution of sodium dichromate and sodium chromate (pH 7.4) was not irritating or corrosive to the eyes of rabbits (Fujii et al. 1976). Histological examination of the eyes of rats exposed to chromium dioxide (15.5 mg chromium(IV)/m^3) in air revealed no lesions (Lee et al. 1989). In a primary eye irritation test, direct conjunctival instillation of 5.2 mg chromium(III) as chromium nicotinate in water to male and female New Zealand albino rabbits produced

conjunctivitis within 1 hour of application, although no corneal opacity or iritis was observed (Shara et al. 2005).

3.2.3.3 Immunological and Lymphoreticular Effects

In addition to the irritating and ulcerating effects, direct skin contact with chromium compounds elicits an allergic response, characterized by eczema or dermatitis, in sensitized individuals. Chromium-induced allergic contact dermatitis is typically isolated to areas at the site of contact, rarely occurring in areas remote to the point of contact (Winder and Carmody 2002). Following an induction phase during which the patient becomes sensitized, subsequent dermal exposure results in an allergic response. The acute response phase lasts for a few days to a few weeks and is characterized by erythema, edema, and small and large blisters; the chronic phase exhibits similar clinical features, but may also include thickened, scaly, and fissured skin (Winder and Carmody 2002). Evaluation by light and electron microscopy of skin biopsies of individuals with active dermatitis due to chromium shows increased intracellular edema of lower epidermal keratinocytes, formation of vacuoles in cells of the lower epidermis and dendritic, spindle-shaped cells in the upper dermis (Shah and Palmer 2002).

Studies using dermal patch testing as a technique to diagnose chromium sensitivity show that challenge with small amounts of chromium(VI) or chromium(III) can induce a response in sensitized individuals. A series of studies conducted by Hansen et al. (2003, 2006a, 2006b) show that patients with chromium-induced dermatitis associated with exposure to leather products responded to both low-dose and high-dose chromium(VI) and chromium(III) challenge using skin patch tests. In a group of 18 patients previously diagnosed with chromium sensitivity, the concentration of chromium(VI) as potassium dichromate required to elicit a positive response on skin patch challenge was 6 mg chromium(VI)/L and 1 mg chromium(III)/L as chromium trichloride (Hansen et al. 2003). Using higher doses in 2,211 patients with suspected contact dermatitis, 71 (3.2%) tested positive to 0.5% potassium dichromate (0.18% chromium(VI)) on skin patch challenge; of these 71 chromium(VI)-positive patients, 31 also produce a positive result when challenged with 13% chromium trichloride (3.7% chromium(III)) (Hansen et al. 2006b). The positive response to both chromium(VI) and chromium(III) challenge may indicate that exposure to both compounds may induced sensitivity or that there is cross-sensitivity between chromium(VI) and chromium(III) compounds on challenge. Similar results have been reported with high-dose chromium(III), showing that patch testing of chromium(VI)-sensitive patients with chromium(III) compounds can elicit an allergic reaction (Fregert and Rorsman 1964, 1966; Mali et al. 1966).

A study was performed on 54 volunteers who with chromium-induced allergic contact dermatitis to determine a dose-response relationship and to determine a minimum-elicitation threshold concentration (MET) that produces an allergic response in sensitive individuals (Nethercott et al. 1994). Patch testing was performed on the subjects in which the concentration of potassium chromate(VI) was varied up to 4.4 μg chromium/cm^2. Two percent (1/54) had a MET of 0.018. About 10% were sensitized at 0.089 μg/cm^2 and all were sensitized at 4.4 μg/cm^2. Comparable studies were performed with chromium(III) chloride, however, only 1 showed a positive response at 33 μg chromium/cm^2, and upon retesting was negative. Based on these findings the authors concluded that soil concentrations of chromium(VI) and chromium(III) of 450 and 165,000 ppm, respectively, should not pose a hazard of allergic contact dermatitis to 99.99% of people who might be exposed to chromium through soil-skin contact.

Numerous studies have investigated the cause of dermatitis in patients and in workers in a variety of occupations and industries and have determined that chromium compounds are the sensitizing agents. In these studies, patch tests were conducted with chromium(VI) or chromium(III) compounds using various concentrations. In one study using 812 healthy volunteers, patch testing with a 0.5% solution of potassium dichromate chromium(VI) revealed chromium sensitivity in 14 of the volunteers (1.7% of the test population). Of the 14 positive reactions, 10 occurred in a group of 110 offset printers, lithographers, and printing plant cleaners with concurrent exposure to chromium (Peltonen and Fraki 1983). Subjects with a sensitivity to chromium and challenged with a 0.001% solution potassium dichromate had increased skin thickness and blood flow (Eun and Marks 1990). Studies conducted on chromium(VI) sensitive printers and lithographers indicate that chromium(VI) compounds elicit reactions more frequently than do chromium(III) compounds (Levin et al. 1959; Mali et al. 1966; Samitz and Shrager 1966). The authors attributed this to a greater degree of permeation of the hexavalent form than the trivalent form through the skin (see Section 3.4.1.3).

In a study of skin disease among workers at an automobile factory, 230 workers with skin disease and 66 controls were patch tested with potassium dichromate (0.175% chromium(VI)). Sensitivity to potassium dichromate was seen in 24% of the patients and 1% of the controls. Most of the sensitive patients were assemblers who handled nuts, bolts, screws, and washers, which were found to have chromate on the surfaces as a result of a chromate dip used in the engine assembly process. Discontinuation of use of the chromate dip resulted in a significant decrease in the prevalence of dermatitis 6 months later (Newhouse 1963). Among 300–400 men directly exposed to cement dust, 8 had clinical symptoms of cement eczema. All eight tested positive with potassium dichromate, while only

four tested positive with cement (Engebrigtsen 1952). Patch testing of employees of the Baltimore and Ohio Railroad system with a variety of chemicals revealed that in 32 of 98 cases of dermatitis, the antirust diesel-engine coolant compound, which contained sodium chromate, was the etiological agent (Kaplan and Zeligman 1962). Among 200 employees who worked in a diesel locomotive repair shop, 6 cases of chromate dermatitis were diagnosed by positive patch tests to samples of radiator fluid and to 0.25% sodium dichromate (0.09% chromium(VI)). The radiator fluid to which the workers were occupationally exposed contained 66% sodium dichromate (Winston and Walsh 1951). A search for the source of chromium exposure in workers who developed contact dermatitis in wet sandpapering of primer paint on automobiles revealed that the paint contained zinc chromate (Engel and Calnan 1963).

In a study of 1,752 patients considered to have occupational dermatoses, contact dermatitis was the main diagnosis in 1,496 patients (92% women, 83% men). The allergic type, as opposed to the irritant type, was more prevalent in men (73%) than in women (51%). Positive patch tests to chromium (not otherwise specified) occurred in 8% of the women and 29% of the men. Among 280 chromium-sensitized men, 50% were employed in building and concrete work, 17% in metal work, and 12% in tanneries. In the 42 chromium-sensitized women, 20% were in cement work, 19% in metal work, 28% in cleaning, and 15% in laboratory work (Fregert 1975). A survey study of 335 construction workers (including tile setters, painters, construction and cement workers, and wood processors) with occupational dermatitis showed that 152 workers (approximately 45%) were sensitized to chromium based on positive to patch test to potassium dichromate (Bock et al. 2003).

Chromate sensitivity has also been reported in women who frequently used dichromate-containing detergent and bleach (Basketter et al. 2001; Wahba and Cohen 1979).

Other industries and sources of chromium that have resulted in chromium sensitivity include welding, printing, glues, wood ash, foundry sand, match heads, machine oils, timber preservative, boiler linings, making of television screens, magnetic tapes, tire fitting, chrome plating, wood and paper industry, leather tanning, cement working, and milk testing (Burrows 1983; Chen et al. 2008; Gass and Todd 2007; Lockman 2002; Wong et al. 1998).

Animals can also be sensitized to chromium compounds. Contact sensitivity was induced in mice by rubbing a solution of 1% potassium dichromate (0.35% chromium(VI)) ≈50 times on the shaved abdomens. Challenge with potassium dichromate on the ear resulted in significant induction of

sensitivity, measured by ear thickness and histologically observed infiltration of nucleophilic leukocytes (Mor et al. 1988).

Guinea pigs can be sensitized to chromium(VI) and chromium(III) compounds by a series of intradermal injections of 0.009 mg chromium(VI)/kg as potassium dichromate or of 0.004 mg chromium(III)/kg as chromium trichloride. Regardless of the compound used to sensitize the guinea pigs, subsequent patch testing with chromium(VI) or chromium(III) yielded the same erythmatic reaction. The response, however, was greater when chromium(VI) was used as the sensitizer (Gross et al. 1968). Similarly, the same erythmatic response to chromium(VI) and chromium(III) compounds was noted in guinea pigs sensitized to 0.04 mg chromium(VI)/kg as potassium dichromate or 0.03 mg chromium(III)/kg as chromium sulfate (Jansen and Berrens 1968).

Results of skin testing to demonstrate or diagnose chromium sensitization are recorded in Table 3-5 for chromium(VI) and Table 3-6 for chromium(III).

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

3.2.3.4 Neurological Effects
3.2.3.5 Reproductive Effects
3.2.3.6 Developmental Effects

3.2.3.7 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to chromium compounds.

3.3 GENOTOXICITY

In vivo studies of chromium compounds are summarized in Table 3-7. *In vitro* studies on the genotoxicity of chromium(VI) and chromium(III) compounds are summarized in Tables 3-8 and 3-9, respectively. Chromium(VI) compounds rapidly (within seconds to minutes) enter cells by facilitated diffusion, while chromium(III) compounds enter much more slowly (within days) by simple diffusion; therefore, chromium(VI) compounds are of greater concern with regard to health effects. Available genotoxicity studies on occupationally exposed humans typically evaluate effects in blood cells since blood is easily accessible, whereas evaluation of effects in cells from cancer target tissues (e.g., lung,

Table 3-7. Genotoxicity of Chromium *In Vivo*

Species (test system)	End point	Results	Reference	Valence	Compound
Drosophila melanogaster	Gene mutation	+	Gava et al. 1989b; Rasmuson 1985; Rodriguez-Arnaiz and Martinez 1986; Zimmering et al. 1985	(VI)	Potassium dichromate, sodium dichromate, chromium trioxide, calcium chromate
D. melanogaster	Gene mutation	+	Olvera et al. 1993	(VI)	Chromium trioxide
D. melanogaster	Gene mutation	+	Kaya et al. 2002	(VI)	Potassium dichromate
D. melanogaster	Gene mutation	+	Amrani et al. 1999	(VI)	Potassium chromate, potassium dichromate
D. melanogaster	Gene mutation	–	Amrani et al. 1999	(III)	Chromium chloride
Human lymphocytes	Chromosomal aberrations	+	Koshi et al. 1984; Sarto et al. 1982	(VI)	Stainless steel, welding fumes, chromium trioxide
Human lymphocytes	Chromosomal aberrations	–	Hamamy et al. 1987	(III)	Chrome alum (primarily chromium sulfate)
Human lymphocytes	Chromosomal aberrations	–	Husgafvel-Pursiainen et al. 1982	(VI)	Stainless steel, welding fumes
Human lymphocytes	Sister chromatid exchanges	+	Koshi et al. 1984; Lai et al. 1998; Sarto et al. 1982; Stella et al. 1982	(VI)	Chromium plating, stainless steel, welding fumes, chromium trioxide
Human lymphocytes	DNA strand breaks, hydroxylation of deoxyquanosine	–	Gao et al. 1994	(VI)	Production of bichromate
Human lymphocytes	Sister chromatid exchanges	–	Nagaya et al. 1991	(VI)	Chromium plating
Human lymphocytes	Sister chromatid exchanges, DNA strand breaks	+	Werfel et al. 1998	(VI)	Welding fumes
Human lymphocytes	Sister chromatid exchanges	–	Nagaya 1986	(VI)	Chromium plating

Table 3-7. Genotoxicity of Chromium *In Vivo*

Species (test system)	End point	Results	Reference	Valence	Compound
Human peripheral lymphocytes	Micronuclei	+	Vaglenov et al. 1999	(VI)	Chromium electroplating
Human peripheral lymphocytes	Micronuclei	+	Benova et al. 2002	(VI)	Chromium plating
Human buccal mucosa	Micronuclei	+	Benova et al. 2002	(VI)	Chromium plating
Human peripheral lymphocytes	Chromosome aberrations, sister chromatid exchanges	–	Benova et al. 2002	(VI)	Chromium plating
Human peripheral lymphocytes	DNA strand breaks	+	Gambelunghe et al. 2003	(VI)	Chromium plating
Human buccal mucosa	Chromosome aberrations, sister chromatid exchanges	–	Benova et al. 2002	(VI)	Chromium plating
Human whole blood cells	Sister chromatid exchanges	+	Wu et al. 2001	(VI)	Chromium electroplating
Human peripheral lymphocytes	Micronuclei, DNA-protein crosslinks	+	Medeiros et al. 2003a	(III)	Tanners
Human peripheral lymphocytes	Micronuclei	–	Medeiros et al. 2003a	(VI)	Welders
Human peripheral lymphocytes	DNA-protein crosslinks	+	Medeiros et al. 2003a	(VI)	Welders
New polychromatic erythrocytes	Micronuclei	+	LeCurieux et al. 1992	(VI)	Potassium chromate
Rat lung (intratracheal exposure)	DNA alterations	+	Izzotti et al. 1998	(VI)	Sodium dichromate
Rat liver (intratracheal exposure)	DNA alterations	–	Izzotti et al. 1998	(VI)	Sodium dichromate
Rat liver (oral exposure)	DNA-protein crosslinks	+	Coogan et al. 1991a	(VI)	Potassium chromate
Rat liver and kidney nuclei (intraperitoneal exposure)	DNA crosslinks, DNA-protein crosslinks, DNA strain breaks	–	Cupo and Wetterhahn 1985	(III)	Chromium oxide
Rat liver, kidney, and lung nuclei (intraperitoneal exposure)	DNA-protein crosslinks	+	Tsapalos et al. 1983b	(VI)	Sodium dichromate
Rat hepatocytes (oral exposure)	Unscheduled DNA synthesis	–	Mirsalis et al. 1996	(VI)	Potassium chromate
Rat (F344/N) bone marrow cells (oral exposure)	Micronuclei	–	NTP 2008b	(III)	Chromium picolinate

Table 3-7. Genotoxicity of Chromium *In Vivo*

Species (test system)	End point	Results	Reference	Valence	Compound
Rat (Sprague-Dawley) hepatic	DNA fragmentation	–	Shara et al. 2005	(III)	Niacin-bound chromium
Mouse erythrocytes (oral exposure)	Micronuclei	–	Shindo et al. 1989	(VI)	Potassium chromate
Mouse ($B_6C_3F_1$, BALB/c) erythrocytes (oral exposure)	Micronuclei	–	NTP 2007	(VI)	Sodium dichromate dihydrate
Mouse (am3-C57BL/6) erythrocytes (oral exposure)	Micronuclei	+	NTP 2007	(VI)	Sodium dichromate dihydrate
Mouse $B_6C_3F_1$ (oral exposure)	Micronuclei	–	NTP 2008b	(III)	Chromium picolinate monohydrate
Mouse ($B_6C_3F_1$) erythrocytes (oral exposure)	Micronuclei	–	NTP 2008b	(III)	Chromium picolinate monohydrate
Mouse (transplacental exposure)	DNA deletions	+	Kirpnick-Sobol et al. 2006	(III)	Chromium (III) chloride salt
Mouse (transplacental exposure)	DNA deletions	+	Kirpnick-Sobol et al. 2006	(VI)	Potassium dichromate
Mouse (BDF1) bone marrow cells (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (BDF1) peripheral blood cells (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (BDF1) bone marrow cells (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (BDF1) peripheral blood cells (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (BDF1) peripheral blood cells (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(III)	Chromic potassium sulfate dodecahydrate
Mouse (BDF1) bone marrow cells (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(III)	Chromic potassium sulfate dodecahydrate
Mouse (BDF1) bone marrow cells (gavage exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Potassium dichromate

Table 3-7. Genotoxicity of Chromium *In Vivo*

Species (test system)	End point	Results	Reference	Valence	Compound
Mouse (BDF1) bone marrow cells (intraperitoneal exposure)	Micronuclei	+	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (Swiss) bone marrow—dams (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (Swiss) bone marrow—dams (drinking water exposure)	Micronuclei	–	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (Swiss) bone marrow—dams (intraperitoneal exposure)	Micronuclei	+	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (Swiss) bone marrow—dams (intraperitoneal exposure)	Micronuclei	+	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (Swiss) fetal liver cells (transplacental exposure from drinking water)	Micronuclei	–	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (Swiss) fetal liver cells (transplacental exposure from drinking water)	Micronuclei	–	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (Swiss) fetal peripheral blood cells (transplacental exposure from drinking water)	Micronuclei	–	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (Swiss) fetal peripheral blood cells (transplacental exposure from drinking water)	Micronuclei	–	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (Swiss) fetal liver cells (transplacental exposure from intraperitoneal injection)	Micronuclei	+	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate
Mouse (Swiss) fetal liver cells (transplacental exposure from intraperitoneal injection)	Micronuclei	+	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse (Swiss) fetal peripheral blood cells (transplacental exposure from intraperitoneal injection)	Micronuclei	+	De Flora et al. 2006	(VI)	Sodium dichromate dihydrate

Table 3-7. Genotoxicity of Chromium *In Vivo*

Species (test system)	End point	Results	Reference	Valence	Compound
Mouse (Swiss) fetal peripheral blood cells (transplacental exposure from intraperitoneal injection)	Micronuclei	+	De Flora et al. 2006	(VI)	Potassium dichromate
Mouse leukocytes	DNA damage	+	Devi et al. 2001	(VI)	Potassium dichromate
Mouse erythrocytes (intraperitoneal exposure)	Micronuclei	–	Shindo et al. 1989	(VI)	Potassium chromate
Mouse erythrocytes (intraperitoneal exposure)	Micronuclei	+	Itoh and Shimada 1997; Wild 1978	(VI)	Potassium chromate
Mouse erythrocytes (intraperitoneal exposure)	Micronuclei	–	Itoh and Shimada 1996	(III)	Chromium chloride
Mouse erythrocytes (intraperitoneal exposure)	Micronuclei	+	Itoh and Shimada 1996	(VI)	Potassium chromate
Mouse peripheral lymphocytes	DNA damage	+	Wang et al. 2006	(VI)	Potassium chromate
Mouse bone marrow cells (oral exposure)	Micronuclei	–	Mirsalis et al. 1996	(VI)	Potassium chromate
Mouse bone marrow cells (gavage)	Chromosomal aberrations	+	Sarkar et al. 1993	(VI)	Chromium trioxide
Mouse bone marrow (intraperitoneal exposed)	Cell mutation	+	Itoh and Shimada 1998	(VI)	Potassium dichromate
Mouse hepatocytes (intraperitoneal exposed)	Cell mutation	+	Itoh and Shimada 1997, 1998	(VI)	Potassium dichromate
Mouse bone marrow (intraperitoneal exposed)	Micronuclei	+	Chorvatovičová et al. 1993; Wrońska-Nofer et al. 1999	(VI)	Potassium dichromate
Mouse (intraperitoneal exposure)	Dominant lethality	+	Paschin et al. 1982	(VI)	Potassium dichromate
Mouse liver and kidney cells (intraperitoneal exposure)	Single strand breaks	+	Ueno et al. 2001	(VI)	Potassium dichromate
Mouse spleen, lung, and brain cells (intraperitoneal exposure)	Single strand breaks	–	Ueno et al. 2001	(VI)	Potassium dichromate

– = negative results; + = positive results; (0) = 0 valence; (III) = trivalent; (VI) = hexavalent; DNA = deoxyribonucleic acid;

Table 3-8. Genotoxicity of Chromium(VI) *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Compound
Subcellular targets:					
Escherichia coli DNA	DNA-protein crosslinks	No data	–	Fornance et al. 1981	Potassium chromate
Nuclei of mouse L1210 leukemia cells	DNA fragmentation	No data	–	Fornance et al. 1981	Potassium chromate
Double-standed M13mp2 bacteriophage DNA transferred to *E. coli*	Forward mutations	No data	+	Snow and Xu 1989	Potassium chromate
Puc 19 plasmid DNA	Gene mutation	No data	+	Kortenkamp et al. 1996b	Potassium chromate
Papilloma virus	Gene mutation	No data	+	Kowalski et al. 1996	Potassium chromate
PSV2neo-based plasmid DNA	DNA polymerase arrest	+	–	Bridgewater et al. 1994b, 1998	Sodium dichromate
Prokaryotic organisms:					
Bacillus subtilis	Recombinations	No data	+	Kanematsu et al. 1980; Nakamuro et al. 1975	Potassium chromate, potassium dichromate
E. coli PQ37, PQ35	Induction of SOS response	–	+	Olivier and Marzin 1987	Potassium chromate, potassium dichromate
E. coli AB1157, GC2375, UA4202, PQ30	Induction of SOS response	No data	+	Llagostera et al. 1986	Chromium chromate, potassium dichromate, chromium trioxide
E. coli Wp2, Hs30R, B/rWP2	Reverse mutations	No data	+	Kanematsu et al. 1980; Nakamuro et al. 1978; Venitt and Levy 1974	Potassium dichromate, potassium chromate, sodium chromate

Table 3-8. Genotoxicity of Chromium(VI) *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
E. coli, WP2/pKM101, WP2 uvrA/pKM101	Reverse mutations	No data	+	Watanabe et al. 1998a	Chromium trioxide, sodium dichromate
E. coli, WP2 uvrA/pKM101	Reverse mutations	+	+	NTP 2007	Sodium dichromate dihydrate
Salmonella typhimurium TA100, TA98	Reverse mutations	+	+	NTP 2007	Sodium dichromate dihydrate
S. typhimurium TA100	Base pair substitutions	No data	+	DeFlora 1978	Sodium dichromate
S. typhimurium TA100	Base pair substitutions	No data	+	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA102	Base pair substitutions	No data	+	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA92	Base pair substitutions	No data	+	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA1535	Base pair substitutions	No data	–	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA97	Frame shift mutations	No data	+	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA1537, TA1538	Frame shift mutations	No data	–	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA1978	Frame shift mutations	No data	±	Bennicelli et al. 1983	Sodium dichromate
S. typhimurium TA1535	Base pair substitutions	–	±	Nakamura et al. 1987	Potassium dichromate
S. typhimurium TA100	Base pair substitutions	+	+	Venier et al. 1982	Potassium dichromate
S. typhimurium TA1538	Frame shift mutations	–	–	Venier et al. 1982	Potassium dichromate
S. typhimurium TA98	Frame shift mutations	–	±	Venier et al. 1982	Potassium dichromate
S. typhimurium TA97a, TA98	Frame shift mutations	+	+	Tagliari et al. 2004	Potassium dichromate
S. typhimurium TA100, TA102	Base pair substitutions	+	+	Tagliari et al. 2004	Potassium dichromate

Table 3-8. Genotoxicity of Chromium(VI) *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
S. typhimurium TA100	Base pair substitutions	–	–	DeFlora 1981	Sodium dichromate, potassium chromate, calcium chromate, ammonium chromate, chromium trioxide
S. typhimurium TA1535	Base pair substitutions	No data	+	DeFlora 1981	Sodium dichromate, potassium chromate, calcium chromate, ammonium chromate, chromium trioxide
S. typhimurium TA100, TA1535	Base pair substitutions	No data	+	Haworth et al. 1983	Calcium chromate
S. typhimurium TA98, TA1537	Frame shift mutations	No data	+	Haworth et al. 1983	Calcium chromate
S. typhimurium TA100, TA1535	Base pair substitutions	No data	–	Kanematsu et al. 1980	Potassium dichromate
S. typhimurium TA100, TA1537, TA1538	Frame shift mutations	No data	–	Kanematsu et al. 1980	Potassium dichromate
S. typhimurium TA 1535 pSK1002	Mutations	+	+	Yamamoto et al. 2002	Potassium dichromate
S. typhimurium TA102, TA2638	Reverse mutations	No data	+	Watanabe et al. 1998a	Chromium trioxide, sodium dichromate
Eukaryotic organisms:					
Yeasts:					
Saccharomyces cerevisiae D7	Mitotic gene conversions	No data	+	Fukunaga et al. 1982; Singh 1983	Chromium trioxide
S. cerrevisiae D7	Reverse mutations	No data	+	Singh 1983	Potassium dichromate
S. cerrevisiae D7	Mitotic cross-over	No data	+	Fukunaga et al. 1982	Chromium trioxide
S. cerrevisiae	DNA deletions	No data	+	Kirpnick-Sobol et al. 2006	Potassium dichromate

Table 3-8. Genotoxicity of Chromium(VI) *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
Schizosacharomyces pombe	Mitotic gene conversion	No data	+	Bonatti et al. 1976	Potassium dichromate
S. pombe	Forward mutations	No data	+	Bonatti et al. 1976	Potassium dichromate
Chickens:					
Chick embryos	DNA damage cross links, strand breaks, DNA-protein crosslinks	No data	+	Tsapakos et al. 1983a	Sodium chromate
Mammalian cells:					
Human embryonic lung fibroblasts (IMR-90)	DNA-protein crosslinks, DNA fragmentation	No data	+	Fornance et al. 1981	Potassium chromate
Human bronchial epithelial cells	DNA fragmentation	No data	+	Fornance et al. 1981	Potassium chromate
Human lymphocytes	Single strand breaks	No data	+	Depault et al. 2006	Potassium chromate
Human lymphocytes	DNA damage	No data	+	Blasiak and Kowalik 2000	Potassium dichromate
Human dermal fibroblasts (GM03440 cells)	DNA double-strand breaks	No data	+	Ha et al, 2003, 2004	Sodium chromate
Human bronchial fibroblasts (WTHBF-6 cells)	chromosome aberrations	No data	+	Holmes et al. 2006	Sodium chromate
Human bronchial fibroblasts (WTHBF-6 cells)	Disruption of mitosis	No data	+	Wise et al. 2006a	Sodium chromate
Human bronchial epithelial cells (BEP2D cells)	chromosome aberrations	No data	+	Wise et al. 2006b	Sodium chromate
Human lung fibroblasts	DNA polymerase arrest, DNA-DNA crosslinks	No data	+	Xu et al. 1996	Sodium chromate
Chinese hamster lung DON cells	Sister chromatid exchange, chromosomal aberrations	No data	+	Koshi 1979, Koshi and Iwaski 1983	chromium trioxide, zinc bromate, calcium chromate, potassium chromate
Chinese hamster ovary cells	Chromosomal aberrations, DNA fragmentation	No data	+	Blankenship et al. 1997	sodium chromate

Table 3-8. Genotoxicity of Chromium(VI) *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
Mouse L1210 leukemia cells	DNA fragmentation, DNA-protein crosslinks	No data	+	Fornace et al. 1981	Potassium chromate
Mouse embryo fibroblast cells	Chromosomal aberrations	No data	+	Sugiyama et al. 1986a	Calcium chromate
Mouse A18BcR cells	Unscheduled DNA synthesis	No data	+	Raffetto et al. 1977	Potassium dichromate
Mouse primary fetal cells	Transformations, chromosomal aberrations	No data	+	Raffetto et al. 1977	Potassium dichromate
Human gastric mucosa	DNA damage	No data	+	Trzeciak et al. 2000	Potassium dichromate
Human peripheral blood lymphocytes	DNA damage	No data	+	Trzeciak et al. 2000	Potassium dichromate
Human fibroblasts	Double strand breaks	No data	+	Ha et al. 2004	Sodium chromate
Human primary bronchial fibroblasts	Chromosomal aberrations	No data	+	Wise et al. 2002, 2004	Sodium chromate
Chinese hamster ovary cells	Chromosomal damage	No data	+	Seoane and Dulout 1999	Potassium dichromate
Mouse mammary FM3A carcinoma cells	Chromosomal aberrations	No data	+	Umeda and Nishmura 1979	Potassium dichromate, potassium chromate, chromium trioxide
Rat liver epithelial cells	Transformations	No data	+	Briggs and Briggs 1988	Potassium chromate

– = negative results; + = positive results; ± = weakly positive results; (VI) = hexavalent; DNA = deoxyribonucleic acid

Table 3-9. Genotoxicity of Chromium(III) *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Compound
Subcellular targets:					
Escherichia coli DNA	DNA-protein crosslinks	No data	+	Fornace et al. 1981	Chromium trichloride
Nuclei of mouse L1210 leukemia cells	DNA fragmentation	No data	+	Fornace et al. 1981	Chromium trichloride
Single-stranded M13mp2 bacteriophange DNA	Replication assay: increased nucleotide incorporation	No data	+	Snow 1991; Snow and Xu 1989	Chromium trichloride
Double-stranded M13mp2 bacteriophage DNA transferred to *E. coli*	Forward mutations	No data	+	Snow 1991; Snow and Xu 1989	Chromium trichloride
pSV2neoTS DNA	DNA polymerase arrest	No data	+	Bridgewater et al. 1994b	Chromium trichloride
Prokaryotic organisms:					
Bacillus subtilis	Recombinations	No data	–	Kanematsu et al. 1980	Chromium sulfate, chromium potassium sulfate
B. subtilis	Recombinations	No data	–	Matsui 1980; Nakamuro et al. 1978; Nishioka 1975	Chromium trichloride
B. subtilis	Recombinations	No data	±	Nakamuro et al. 1978	Chromium nitrate
B. subtilis	Recombinations	No data	±	Nakamuro et al. 1978	Chromium acetate
E. coli	Gene mutations	No data	+	Sugden et al. 1990	cis-Dichlorobis (2,2'-bipyridyl) chromium(III)
E. coli WP2 *uvrA*/pKM101	Gene mutations	–	–	NTP 2008b	Chromium picolinate monohydrate
E. coli AB1157, GC275, VA4202, PQ30	Induction of SOS response	No data	–	Llagostera et al. 1986	Chromium trichloride, chromium nitrate, chromium acetate
E. coli PQ37, PQ35	Induction of SOS response	–	–	Olivier and Marzin 1987	Chromium trichloride hexahydrate
E. coli PQ37	Induction of SOS response	–	–	Venier et al. 1989	Chromium trichloride, chromium nitrate

Table 3-9. Genotoxicity of Chromium(III) *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Compound
E. coli PQ37	Induction of SOS response	–	±	Venier et al. 1989	Chromium acetate
Salmonella typhimurium TA100, TA1535	Reverse mutationsBase pair substitutions	–	–	De Flora 1981;Petrilli and De Flora 1978b	Chromium trichloride hexahydrate, chromium nitrite, monohydrate, chromium potassium sulfate, chromium acetate, neochromium, chromium alum, chromite
TA98, TA1537, TA1538	Frame shift mutations	–	–		
S. typhimurium TA102	Base pair substitutions	–	–	Bennicelli et al. 1983	Chromium nitrate
S. typhimurium TA100, TA1535	Base pair substitutions	–	–	Venier et al. 1982	Chromium chloride hexahydrate, chromium nitrate monohydrate
TA98, TA1538	Frame shift mutations	–	–		
S. typhimurium TA100, TA98	Reverse mutations	–	–	NTP 2008b	Chromium picolinate monohydrate
S. typhimurium TA102, TA104, TA100, TA1535, TA97, TA98	Reverse mutations	–	–	NTP 2008b	Chromium picolinate
S. typhimurium TA92, TA98, TA100	Reverse mutations	No data	+	Warren et al. 1981	Chromium complexes with 2,2'-bipyridine and 1,10-phenanthroline
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutations	–	–	Whittaker et al. 2005	Chromium picolinate
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutations	–	–	Whittaker et al. 2005	Chromium chloride
S. typhimurium TA1535, TA97a, TA98, TA100, TA102	Reverse mutations	–	–	Shara et al. 2005	Niacin-bound chromium
S. typhimurium TA 1535 pSK1002	Mutations	–	–	Yamamoto et al. 2002	Chromium nitrate

Table 3-9. Genotoxicity of Chromium(III) *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
Eukaryotic organisms:					
Yeasts:					
Saccharomyces cerevisiae	Reverse mutations, mitotic gene conversions	No data	+	Bronzetti et al. 1986	Chromium trichloride
S. cerrevisiae	DNA deletions	No data	+	Kirpnick-Sobol et al. 2006	Cromium (III) chloride salt
Chickens:					
Chick embryos	DNA damage (crosslinks, strand breaks)	No data	–	Tsapakos et al. 1983a	Chromium nitrate
Mammalian cells:					
Human lymphocytes	DNA damage	No data	+	Blasiak and Kowalik 2000	Chromium chloride
Human skin fibroblasts	Unscheduled DNA synthesis	No data	–	Whiting et al. 1979	Chromium trichloride
Human skin fibroblasts	DNA fragmentation	No data	–	Whiting et al. 1979	Chromium trichloride
Human leukocytes	Chromosomal aberrations	No data	±	Nakamuro et al. 1978	Chromium trichloride, chromium nitrate, chromium acetate
Human lymphocytes	Chromosomal aberrations	No data	±	Stella et al. 1982	Chromium trichloride hexahydrate
Human lymphocytes	Chromosomal aberrations	No data	–	Sarto et al. 1980	Chromium trichloride
Human lymphocytes	Sister chromatid exchange	No data	–	Stella et al. 1982	Chromium trichloride hexahydrate
Chinese hamster V79 cells	Chromosomal aberrations	No data	–	Newbold et al. 1979	Chromium acetate
Syrian hamster embryonal cells	Chromosomal aberrations	No data	–	Tsuda and Kato 1977	Chromium trichloride hexachloride, chromium sulfate tetrahydrate
Chinese hamster lung DON cells	Chromosomal aberrations	No data	–	Ohno et al. 1982	Chromium trichloride hexahydrate, chromium sulfate tetrahydrate

Table 3-9. Genotoxicity of Chromium(III) *In Vitro*

Species (test system)	End point	Results		Reference	Compound
		With activation	Without activation		
Chinese hamster ovary cells	aberrations		±	Levis and Majone 1979	Chromium trichloride hexachloride, chromium nitrate monohydrate, chromium potassium sulfate, chromium acetate
Chinese hamster ovary cells	Sister chromatid exchange	No data	–	Levis and Majone 1979; MacRae et al. 1979; Venier et al. 1982	Chromium trichloride hexachloride, chromium nitrate, monohydrate, chromium potassium sulfate, chromium acetate
Chinese hamster ovary cells (*hprt* locus)	Mutations	No data	+	Coryell and Stearns 2006; Stearns et al. 2002	Chromium trispicolinate
Mouse L5178Y+/- lymphoma	Mutations	–	–	Shara et al. 2005	Niacin-bound chromium
Mouse L5178Y lymphoma	Mutations	+	+	Whittaker et al. 2005	Chromium picolinate
Mouse L5178Y lymphoma	Mutations	–	±	Whittaker et al. 2005	Chromium chloride
Mouse leukemia cells	Chromosomal aberrations	No data	–	Fornace et al. 1981	Chromium trichloride
Mouse mammary carcinoma	Chromosomal aberrations	No data	–	Umeda and Nishimura 1979	Chromium sulfate
Fm3A cells:					
Mouse fetal cells	Chromosomal aberrations	No data	±	Raffetto et al. 1977	Chromium trichloride
Mouse fetal cells	Morphological transformations	No data	+	Raffetto et al. 1977	Chromium trichloride
Mouse A18BcR cells	Unscheduled DNA synthesis	No data	–	Raffetto et al. 1977	Chromium trichloride

– = negative results; + = positive results; ± = weakly positive results; (III) = trivalent; DNA = deoxyribonucleic acid

gastrointestinal tract) are not easily obtained for analysis. However, negative genotoxicity results in tissues that are not cancer targets (e.g., blood) should not be extrapolated to cancer target tissues.

Occupational exposure studies have yielded mixed results on the genotoxic potential of chromium compounds. Studies involving workers exposed to chromium(VI) in stainless steel welding and electroplating (Husgafvel-Pursiainen et al. 1982; Littorin et al. 1983; Nagaya 1986; Nagaya et al. 1991), and to chromium(III) in tanneries (Hamamy et al. 1987) did not report increases in the number of chromosomal aberrations or sister chromatid exchanges in peripheral lymphocytes of these workers. No elevations in DNA strand breaks or hydroxylation of deoxyguanosine were detected in lymphocytes of workers exposed to chromium(VI) involved in the production of bichromate (Gao et al. 1994), while DNA strand breaks were reported in the peripheral lymphocytes of 19 chromium platers (Gambelunghe et al. 2003). In contrast, other studies involving electroplaters and stainless steel welders reported higher levels of chromosomal aberrations or sister chromatid exchanges in workers exposed to chromium(VI) compared to controls (Deng et al. 1988; Koshi et al. 1984; Lai et al. 1998; Sarto et al. 1982; Stella et al. 1982; Werfel et al. 1998).

Urine samples from six workers working in chromium plating factories were tested for the induction of unscheduled DNA synthesis (UDS) in pleural mesothelial cells (Pilliere et al. 1992). The mean chromium concentration in the urine samples was 11.7±8.8 μg/L. The urine from five of the workers showed a significant elevated in UDS over control subjects who were nonsmokers, with a trend toward increasing amounts of urine being tested. However, there was no correlation between UDS and chromium concentrations in urine.

An epidemiology study of stainless steel welders, with mean exposure levels of 0.055 mg chromium(VI)/m^3 or 0.081 mg chromium (total)/m^3, did not report increases in the number of sister chromatid exchanges in the lymphocytes of exposed workers. The welders were also exposed to nickel and molybdenum from the welding rods (Littorin et al. 1983). A similar study was conducted to detect genotoxic effects of chromium(VI) on workers in electroplating factories. Of the 24 workers examined, none showed significant differences in sister chromatid exchange frequency (Nagaya 1986). Similarly, no correlation was found between excretion of chromium in the urine and the frequency of sister chromatid exchanges in 12 male chromium platers whose mean urinary chromium level was 17.9 μg/g creatinine (Nagaya et al. 1991). In chrome platers (n=15) in low (0.0075 mg Cr(VI)/m^3) and high (0.0249 mg Cr(VI)/m^3) exposure groups, no significant differences in the frequency of sister chromatid exchanges and chromosomal aberrations in peripheral lymphocytes and buccal mucosa cells were observed

compared to controls (0.0004 mg Cr(VI)/m^3; n=15) (Benova et al. 2002). No increase in chromosomal aberrations was observed in 17 tannery workers exposed primarily to chromium(III) as compared with 13 controls (Hamamy et al. 1987). However, parallel measurements in these tannery workers showed that the average chromium levels in plasma (0.115 μg/L) and urine (0.14 μg/100 L) did not differ from the nonexposed workers. In addition, stainless steel welders occupationally exposed to chromium(VI) for a mean of 21 years did not have any increase in chromosomal aberrations or sister chromatid exchanges compared to a control group. No actual exposure levels were provided (Husgafvel-Pursiainen et al. 1982). Yet, other studies involving electroplaters and welders report a higher incidence of chromosomal aberrations or sister chromatid exchanges in lymphocytes of workers than in controls. In one study, a causal relationship between chromium exposure and the observed effects could not be established because the exposure was confounded by co-exposure to nickel and manganese (Elias et al. 1989a). In another study, although chromium workers were found to have higher rates of sister chromatid exchanges than workers exposed to nickel-chromium or controls (after adjusting for potential confounding factors), the differences were not significantly correlated to chromium concentrations in blood or urine (Lai et al. 1998). The frequency of sister chromatid exchanges was also higher in the blood of 35 chromium platers in Taiwan when compared to controls (Wu et al. 2001). The frequency of sister chromatid exchanges in the lymphocytes of 12 workers exposed to chromium(VI) as chromic acid fumes in a chrome plating industry was significantly increased (Stella et al. 1982). Significantly increased incidences of chromosomal aberrations in peripheral lymphocytes were found in workers exposed to chromium(VI) as chromium trioxide in two of four electroplating plants. Of the two plants where the increases were significant, one was a "bright" plating plant, where exposure involved nickel as well as chromium, and one was a "hard" plating plant, where exposure involved only chromium. However, the increase in chromosomal aberrations correlated poorly with urinary chromium levels, and only the increase in the "bright" platers showed a significant correlation with duration of exposure. A significantly increased incidence of sister chromatid exchanges was found in "hard" platers compared with controls (sister chromatid exchange was not evaluated in "bright" platers), and smoking appeared to enhance the increase (7 of 8 smokers and 7 of 11 nonsmokers had incidences significantly higher than controls). Moreover, the increased incidence of sister chromatid exchange showed a positive correlation with urinary chromium levels (Sarto et al. 1982). Repeated cytogenetic analysis of peripheral lymphocytes for 3 years revealed an increased frequency of chromosomal aberrations and sister chromatid exchanges in a group of stainless steel welders compared to controls. The workers were exposed to unreported chromium(VI) concentrations for a mean of 12.1 years, but exposure to ultraviolet rays and small amounts of manganese, nickel, iron, and magnesium could not be ruled out (Koshi et al. 1984). Compared to 39 controls, significantly elevated sister chromatid exchange values in lymphocytes and significantly higher rates of

DNA single-strand breakages were found in a group of 39 welders exposed to unreported chromium(VI) and nickel concentrations (Werfel et al. 1998). Only one study was located regarding the average levels of exposure for electroplating workers: workers exposed to an average level of 0.008 mg chromium(VI)/m^3 had increases in chromosomal aberrations and sister chromatid exchanges. However, high levels of nickel as well as chromium were found in hair and stool samples when compared to controls (Deng et al. 1988). Increased frequencies of micronuclei were reported in the peripheral lymphocytes and buccal mucosa cells in two studies of chromium electroplating workers in Bulgaria (Benova et al. 2002; Vaglenov et al. 1999). In chrome platers (n=15), significant increases in micronuclei in peripheral lymphocytes and buccal mucosa cells were observed in low (0.0075 mg Cr(VI)/m^3) and high (0.0249 mg Cr(VI)/m^3) exposure groups compared to controls (0.0004 mg Cr(VI)/m^3; n=15) (Benova et al. 2002). Increased micronuclei frequency and DNA-protein crosslinks were observed in the peripheral lymphocytes of tanners primarily exposed to chromium(III) compounds, while welders, who are primarily exposed to chromium(VI) compounds had evidence of DNA-protein crosslinks, but not increased micronuclei frequency in peripheral lymphocytes (Medeiros et al. 2003a). No elevated levels of DNA strand breaks or hydroxylation of deoxyguanosine in lymphocytes were found in 10 workers occupationally exposed in the production of bichromate when compared with 10 nonoccupationally-exposed workers at the same facility Gao et al. (1994). From general background monitoring levels of chromium(VI), exposures were estimated to be between 0.001 and 0.055 mg/m^3. In contrast, DNA strand breaks were reported in the peripheral lymphocytes of 19 chromium platers with a mean postshift urinary concentration of 7.31 μg/g creatinine when compared to non-exposed control subjects (Gambelunghe et al. 2003).

Chromium(VI) and chromium(III) have been shown to be genotoxic in human cell lines. S phase-dependent DNA double-strand breaks were observed in cultured human dermal fibroblasts exposed to sodium chromate (chromium(VI)) (Ha et al. 2003, 2004). Sodium chromate also induced concentration-dependent chromosome damage in cultured human bronchial fibroblasts and bronchial epithelial cells (Holmes et al. 2006; Wise et al. 2006b). Exposure of cultured human bronchial fibroblasts to sodium chromate produced disruption of mitosis, most likely through spindle assembly checkpoint bypass (Wise et al. 2006a). Weakly positive responses were observed for chromium(III) (Nakamuro et al. 1978; Stella et al. 1982). However, it should be noted that in positive studies, the genotoxic potency of chromium(III) compounds was several orders lower than that of chromium(VI) compounds tested in the same systems. Positive results for increased micronuclei and DNA damage were also observed in lymphocytes exposed to chromium(III) chloride (Blasiak and Kowalik 2000). Positive results of chromium(III) in intact cells could be due to contamination of the test compounds with traces of chromium(VI) (De Flora et al. 1990;

IARC 1990), nonspecific effects at very high doses, experimental conditions that would increase the penetration of chromium(III) into cells (e.g., detergents), or a technical artifact formed during the extraction procedures (De Flora et al. 1990). In one case, chromium(III) compounds showed genotoxicity that was linked to redox cycling of a chromium-DNA complex (Sugden et al. 1990). Although chromium(III) compounds are less toxic than chromium(VI) compounds because of its relative inability to cross cell membranes, chromium(III) causes more DNA damage and mutations when it is formed by intracellular reduction from chromium(VI) or it is reacted with DNA in subcellular systems (Bridgewater et al. 1994a, 1994b, 1998; Fornace et al. 1981; Snow 1991; Snow and Xu 1989).

Thus, results of studies in occupationally exposed humans and in human cell lines indicate that chromium(VI) and chromium(III) are genotoxic; however, studies in humans were limited in several aspects. Generally, the levels of exposure to chromium(VI) were not known and co-exposure to other potentially active compounds (namely ultraviolet rays and other potentially genotoxic metals) occurred in several studies. Some negative results (Hamamy et al. 1987) were probably due to low exposure, because the chromium levels in plasma and urine of exposed and unexposed workers did not differ. Furthermore, some of the studies (Deng et al. 1988; Hamamy et al. 1987; Stella et al. 1982) used groups that were too small (<20 individuals) to have the statistical power to reliably assess the cytogenetic changes in workers. Although most older occupational exposure studies gave negative or equivocal results, most recent studies have identified chromosomal effects in exposed workers (Benova et al. 2002; Gambelunghe et al. 2003; Wu et al. 2001). Furthermore, results of studies in human cell lines provide evidence of the genotoxic activity of chromium compounds. Thus, the available studies support that chromium compounds, particularly chromium(VI), have carcinogenic potential because interactions with DNA have been linked with the mechanism of carcinogenicity. No studies were located regarding genotoxic effects in humans after oral exposure to chromium or its compounds.

Numerous studies have evaluated the genotoxicity of chromium compounds in animals by several exposure routes, including oral, inhalation, and parenteral routes. No increased incidence of micronuclei in polychromatic erythrocytes was observed in mice given single gavage doses of potassium chromate at ≤86 mg chromium(VI)/kg (Shindo et al. 1989) or in mice exposed to potassium chromate via drinking water at 1–20 ppm for 48 hours or to bolus doses up to 4 μg/kg for 2 days (Mirsalis et al. 1996). Similarly, no UDS in hepatocytes was found in rats. However, an increase in DNA-protein crosslinking was found in the livers of rats exposed to potassium chromate in the drinking water at ≥6 mg chromium(VI)/kg/day for 3 or 6 weeks (Coogan et al. 1991a).

The clastogenic effects of male Swiss albino mice fed chromium(VI) trioxide (20 mg/kg body weight) by gavage were studied; after 24 hours, bone marrow cells were isolated and 500 metaphase plates were scored for chromosomal aberrations (Sarkar et al. 1993). The treated cells showed a significant increase in aberrations per cell over controls by 4.4-fold. When animals were treated simultaneously with chlorophyllin (1.5 mg/kg), a sodium-copper derivative of chlorophyll and an antioxidant, numbers of aberrations were reduced to nearly background levels.

An increase in DNA-protein crosslinking was found in the livers of rats that had been exposed to potassium chromate in the drinking water at ≥6 mg chromium(VI)/kg/day for 3 or 6 weeks (Coogan et al. 1991a). Bone marrow cells from male mice fed chromium(VI) trioxide at 20 mg chromium(VI)/kg by gavage had a 4.4-fold increase in chromosomal aberration over controls (Sarkar et al. 1993). Significant DNA alterations were seen in the lung, but not the liver, of rats exposed to chromium (VI) by intratrachael instillation of sodium dichromate (Izzotti et al. 1998). DNA damage was also reported in leukocytes and peripheral lymphocytes of mice orally exposed to chromium(VI) as potassium chromate (Devi et al. 2001; Wang et al. 2006), and transplacental exposure of potassium dichromate resulted in DNA deletions the retinal pigment epithelium of mice (Kirpnick-Sobol et al. 2006). Intraperitoneal exposure to chromium(VI) as potassium dichromate caused single strand breaks in mouse liver and kidney cells, but did not in spleen, lung, or brain cells (Ueno et al. 2001). Micronucleated polychromatic erythrocytes were found in mice following intraperitoneal exposure to chromium(VI) as potassium dichromate (Chorvatovičová et al. 1993; De Flora et al. 2006; Itoh and Shimada 1996, 1997; Wild 1978; Wroñska-Nofer et al. 1999), though one study reported negative results following intraperitoneal exposure to potassium chromate (Shindo et al. 1989). In contrast, oral exposure of mice to chromium(VI), as potassium dichromate or sodium dichromate dihydrate, did not induce micronuclei in bone marrow or in peripheral blood cells (De Flora et al. 2006; Mirsalis et al. 1996; NTP 2008a). Similar to chromium(VI) compounds, oral exposure of chromium(III) compounds also did not induce micronuclei in mouse erythrocytes (NTP 2008b), bone marrow cells (De Flora et al. 2006; NTP 2008b), or in peripheral blood cells (De Flora et al. 2006). Transplacental exposure to fetuses from dams exposed to chromium(VI) as either sodium dichromate dihydrate or potassium dichromate through drinking water did not result in micronuclei in fetal liver or peripheral blood cells (De Flora et al. 2006), while transplacental exposure to fetuses from dams exposed by intraperitoneal injection to these same chromium(VI) compounds did result in micronuclei in both fetal liver and peripheral blood cells (De Flora et al. 2006).

No unscheduled DNA synthesis was found in rat hepatocytes after the rats were exposed to potassium chromate in drinking water (Mirsalis et al. 1996). The contrasting results may relate to route-specific

differences in absorption or metabolic fate of chromate *in vivo*. Furthermore, intraperitoneal exposure to chromium(VI) as potassium dichromate induced dominant lethality in mice (Paschin et al. 1982) and a significant increase in mutant frequency within mouse hepatocytes (Itoh and Shimada 1997, 1998) and bone marrow cells (Itoh and Shimada 1998). Intraperitoneal injection in rats with sodium dichromate chromium(VI)) resulted in DNA crosslinks in liver, kidney, and lung nuclei (Tsapakos et al. 1983b), while similar injection in rats with chromium(III) trichloride did not cause DNA interstrand crosslinks, DNA-protein crosslinks, or DNA strand breaks in liver and kidney nuclei (Cupo and Wetterhahn 1985). Oral exposure to niacin-bound chromium(III) did not cause DNA fragmentation in rats after 90 days of dietary exposure at doses >621.6 mg Cr(III)/kg/day (Shara et al. 2005). In addition, studies in *Drosophila melanogaster* showed an induction of gene mutations after exposure to chromium(VI) (Amrani et al. 1999; Gava et al. 1989a; Kaya et al. 2002; Rasmuson 1985; Rodriguez-Arnaiz and Martinez 1986; Olvera et al. 1993; Zimmering et al. 1985), but not after exposure to chromium(III) (Amrani et al. 1999).

The vast majority of studies reported genotoxic effects of chromium(VI) in mammalian cells *in vitro* (Blasiak and Kowalik 2000; Briggs and Briggs 1988; Depault et al. 2006; DiPaolo and Casto 1979; Douglas et al. 1980; Elias et al. 1989b; Fornace et al. 1981; Gomez-Arroyo et al. 1981; Ha et al. 2004; Koshi 1979; Koshi and Iwasaki 1983; Kowalski et al. 1996; Levis and Majone 1979; MacRae et al. 1979; Majone and Levis 1979; Montaldi et al. 1987; Nakamuro et al. 1978; Newbold et al. 1979; Ohno et al. 1982; Raffetto et al. 1977; Sarto et al. 1980; Seoane and Dulout 1999; Stella et al. 1982; Sugiyama et al. 1986a; Trzeciak et al. 2000; Tsuda and Kato 1977; Umeda and Nishimura 1979; Venier et al. 1982; Whiting et al. 1979; Wise et al. 2002, 2003; Yang et al. 1992). Chromium(VI) also induced DNA damage (DNA interstrand crosslinks, DNA strand breaks, DNA-protein crosslinks) in cultured chick embryo hepatocytes (Tsapakos et al. 1983a). In contrast, mostly negative results were reported for chromium(III) in mammalian cells (Fornace et al. 1981; Levis and Majone 1979; MacRae et al. 1979; Newbold et al. 1979; Ohno et al. 1982; Raffetto et al. 1977; Sarto et al. 1980; Shara et al. 2005; Stella et al. 1982; Tsuda and Kato 1977; Umeda and Nishimura 1979; Venier et al. 1982; Whiting et al. 1979) and chick embryo hepatocytes (Tsapakos et al. 1983a). Positive results were obtained in Chinese hamster ovary cells (Coryell and Stearns 2006; Levis and Majone 1979; Stearns et al. 2002), mouse fetal cells (Raffetto et al. 1977), and mouse lymphoma cells (Whittaker et al. 2005). Chromium(III) picolinate caused chromosome damage (Stearns et al. 1995b) and mutations in cultured mammalian cells (Stearns et al. 2002).

Chromium(VI) was genotoxic in *Saccharomyces cerevisiae* (Fukunaga et al. 1982; Kirpnick-Sobol et al. 2006; Singh 1983) and *Schizosaccharomyces pombe* (Bonatti et al. 1976). Two studies demonstrated the genotoxicity of chromium(III) in *S. cerevisiae* (Bronzetti et al. 1986; Kirpnick-Sobol et al. 2006).

In vitro studies indicated that soluble chromium(VI) compounds are mutagenic in *Salmonella typhimurium* reverse mutation assays (Bennicelli et al. 1983; De Flora 1978, 1981; Haworth et al. 1983; Nakamura et al. 1987; NTP 2007a; Venier et al. 1982; Watanabe et al. 1998a; Yamamoto et al. 2002), and in a *Salmonella* microsuspension bioassay (Tagliari et al. 2004). Only one study reported negative results with chromium(VI) in all tested strains (Kanematsu et al. 1980). In contrast, studies with chromium(III) did not report the induction of reverse mutations in *S. typhimurium* (Bennicelli et al. 1983; De Flora 1981; NTP 2008b; Petrilli and De Flora 1978b; Shara et al. 2005; Venier et al. 1982; Whittaker et al. 2005; Yamamoto et al. 2002). After preincubation with mammalian microsomes, the mutagenicity of chromium(VI) compounds was reduced or abolished due to concentrations of the reductant glutathione, cysteine, or NADPH capable of converting chromium(VI) to chromium(III) compounds (Bennicelli et al. 1983; De Flora 1978,1981). Chromium(VI) compounds caused gene mutations in *Bacillus subtilis* (Kanematsu et al. 1980; Nakamuro et al. 1978; Nishioka 1975) and *Escherichia coli* (Kanematsu et al. 1980; Kortenkamp et al. 1996b; Llagostera et al. 1986; Nakamuro et al. 1978; NTP 2007; Olivier and Marzin 1987; Venitt and Levy 1974; Watanabe et al. 1998a). Negative or weakly positive results were reported in *B. subtilis* with chromium(III) (Kanematsu et al. 1980; Matsui 1980; Nakamuro et al. 1978; Nishioka 1975) and mostly negative results were reported in *E. coli* (Llagostera et al. 1986; NTP 2008b; Olivier and Marzin 1987; Venier et al. 1989). However, hydrophobic ligands such as 2,2´-bipyridine, 1,10-phenanthroline, or picolinic acid form complexes with chromium(III), which are able to penetrate cell membranes and to cause genotoxicity. Complexes of chromium(III) with 2,2´-bipyridine or 1,10-phenanthroline were mutagenic in *S. typhimurium* (Warren et al. 1981). Chromium(III) picolinate was not mutagenic in *S. typhimurium* or *E. coli* (NTP 2008b).

A chromium(IV) ester was synthesized with 2,4-dimethyl-pentane-2,4-diol to examined its ability to cause DNA double strand breaks (Luo et al. 1996). Calf thymus DNA was reacted with the chromium(IV) complex (1.3 mg/mL) in the presence of 2 mM hydrogen peroxide for 6 days at pH 6.8. The results showed that the complex in the presence of hydrogen peroxide significantly damaged DNA by causing double strand breaks. Neither chromium(IV) or hydrogen peroxide alone damaged DNA. The kinetics of the reaction of chromium(IV) with hydrogen peroxide showed the formation of proportional amounts of hydroxyl radical with chromium(V). Use of a free radical scavenger prevented DNA strand breaks. Other studies have shown that chromium(IV) is a better Fenton reagent than chromium(V) for

reducing hydrogen peroxide, and thus, chromium(IV)-type damage by generating hydroxyl radicals may also be a contributor of *in vivo* genotoxicity.

In conclusion, chromium(VI) compounds were positive in the majority of tests reported, and their genotoxicity was related to the solubility and, therefore, to the bioavailability to the targets. Results of occupational exposure studies in humans, although somewhat compromised by concomitant exposures to other potential genotoxic compounds, provide evidence of chromium(VI)-induced DNA strand breaks, chromosome aberrations, increased sister chromatid exchange, unscheduled DNA synthesis, and DNA-protein crosslinks. Findings from occupational exposure studies are supported by results of *in vivo* studies in animals, *in vitro* studies in mammalian cells, yeast and bacteria, and studies in cell-free systems. Compared to chromium(VI), chromium(III) was more genotoxic in subcellular targets, but lost this ability in cellular systems. The reduction of chromium(VI) in the cells to chromium(III) and its subsequent genotoxicity may be greatly responsible for the final genotoxic effects (Beyersmann and Koster 1987; Zhitkovich et al. 2005). Reduction of chromium(VI) can also result in the formation of chromium(V), which is highly reactive and capable of interaction with DNA (Jennette 1982; Norseth 1986).

3.4 TOXICOKINETICS

The toxicokinetics of a given chromium compound depend on the valence state of the chromium atom and the nature of its ligands. Naturally occurring chromium compounds are generally in the trivalent state (chromium(III)), while hexavalent chromium compounds (chromium(VI)) are produced industrially by the oxidation of chromium(III) compounds.

The amount and location of deposition of inhaled chromium will be determined by factors that influence convection, diffusion, sedimentation, and interception of particles in the airways. These factors include air flow velocities, which are affected by breathing rate and tidal volume; airway geometry; and aerosol particle size (ICRP 1994). In general, deposition in the thoracic and pulmonary regions of the respiratory tract increase (as a fraction of the total deposited dose) as particle sizes decrease. Larger particles (e.g., >10 μm in diameter) deposit in the extrathoracic region. Chromium that deposits in the respiratory tract are subject to three general clearance processes: (1) mucociliary transport to the gastrointestinal tract for the ciliated airways (i.e. trachea, bronchi, and proximal bronchioles); (2) phagocytosis by lung macrophages and cellular transport to thoracic lymph nodes; or (3) absorption and transfer by blood and/or lymph to other tissues. The above processes apply to all forms of deposited chromium, although

the relative contributions of each pathway and rates associated with each pathway may vary with the physical characteristics (e.g., particle size), chemical form (degree of water solubility), and chemotactic properties of the chromium particles. In general, less water-soluble chromium compounds that deposit in the pulmonary region can be expected to have a longer retention time in the lung than more soluble forms. In addition, lung concentrations of chromium increase with increasing age.

Most quantitative studies of the gastrointestinal absorption of chromium in humans have estimated the absorption fraction to be <10% of the ingested dose. In general, these studies suggest that the absorption fraction of soluble chromium compounds is higher than insoluble forms (e.g., $CrCO_3$), and is higher for soluble chromium(VI) compounds (e.g., $K_2Cr_2O_7$) than soluble chromium(III) (e.g., $CrCl_3$). Chromium(VI) is reduced in the stomach to chromium(III), which lowers the absorbed dose from ingested chromium(VI). Absorption is also affected by the nutritional status of chromium(III); the absorption fraction is higher when dietary intakes are lower. Peak plasma concentrations of chromium occur within 2 hours following an oral dose of soluble chromium, suggesting that absorption occurred. Chromium absorption occurs in the upper small intestine.

Chromium(III) and chromium(VI) can penetrate human skin to some extent, especially if the skin is damaged. Few quantitative estimates of dermal absorption in humans have been reported. A 3-hour immersion in a warm aqueous bath of 22 mg Cr(VI)/L (as $K_2Cr_2O_7$) resulted in absorption (based on urine measurements) of approximately $3.3x10^{-5}$–$4.1x10^{-4}$ μg Cr/cm^2-hour (Corbett et al. 1997).

Absorbed chromium distributes to nearly all tissues, with the highest concentrations found in kidney and liver. Bone is also a major depot and may contribute to long-term retention kinetics or chromium. Chromium(VI) is unstable in the body and is reduced to chromium(V), chromium(IV), and ultimately to chromium(III) by many substances including ascorbate and glutathione. Reduction of chromium(VI) to chromium(III) can give rise to reactive intermediates, chromium adducts with proteins and DNA, and secondary free radicals. Chromium(VI) in blood is taken up into red blood cells, where it undergoes reduction and forms complexes with hemoglobin and other intracellular proteins that are sufficiently stable to retain chromium for a substantial fraction of the red blood cell lifetime. Absorbed chromium can be transferred to fetuses through the placenta and to infants via breast milk. Absorbed chromium is excreted predominantly in urine. Studies in animals have shown that chromium can be secreted in bile following parenteral (e.g., intravenous) injection of chromium(VI) or chromium (III) compounds. Chromium can also be eliminated by transfer to hair and nails. Chromium absorbed following ingestion of chromium(VI) (as $K_2Cr_2O_7$) appears to have a slower elimination rate ($t_{1/2}$ approximately 40 hours)

than when chromium is absorbed following ingestion of soluble chromium(III) (as $CrCl_3$; $t_{1/2}$ approximately 10 hours).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

The absorption of inhaled chromium compounds depends on a number of factors, including physical and chemical properties of the particles (oxidation state, size, solubility) and the activity of alveolar macrophages.

The identification of chromium in urine, serum and tissues of humans occupationally exposed to soluble chromium(III) or chromium(VI) compounds in air indicates that chromium can be absorbed from the lungs (Cavalleri and Minoia 1985; Gylseth et al. 1977; Kiilunen et al. 1983; Mancuso 1997b; Minoia and Cavalleri 1988; Randall and Gibson 1987; Tossavainen et al. 1980). In most cases, chromium(VI) compounds are more readily absorbed from the lungs than chromium(III) compounds, due in part to differences in the capacity to penetrate biological membranes. Nevertheless, workers exposed to chromium(III) lignosulfonate dust at 0.005–0.23 mg chromium(III)/m^3 had clearly detectable concentrations of chromium in the urine at the end of their shifts. Based on a one-compartment kinetic model, the biological half-life of chromium(III) from the lignosulfonate dust was 4–10 hours, which is the same order of magnitude as the half-life for chromium(VI) compounds (Kiilunen et al. 1983).

Rats exposed to 2.1 mg chromium(VI)/m^3 as zinc chromate 6 hours/day achieved steady-state concentrations in the blood after ~4 days of exposure (Langård et al. 1978). Rats exposed for a single inhalation of chromium(VI) trioxide mist from electroplating at a concentration of 3.18 mg chromium(VI)/m^3 for 30 minutes rapidly absorbed chromium from the lungs. The content of chromium in the lungs declined from 13.0 mg immediately after exposure to 1.1 mg at 4 weeks in a triphasic pattern with an overall half-life of 5 days (Adachi et al. 1981). Based on a study in rats exposed to chromium(VI) as potassium dichromate or to chromium(III) as chromium trichloride, the pulmonary clearance of both valence states was dependent on particle size, and chromium(VI) was more rapidly and extensively transported to the bloodstream than chromium(III). The rats had been exposed to 7.3–15.9 mg chromium(VI)/m^3 as potassium dichromate for 2–6 hours or to 8 or 10.7 mg chromium(III)/m^3 as chromium trichloride for 6 or 2 hours, respectively. Chromium(VI) particles of 1.5 or 1.6 μm had a two-compartment pulmonary clearance curve with half-lives of 31.5 hours for the first phase and 737 hours for the second phase. Chromium(VI) particles of 2 μm had a single component curve with a half-life

between 151 and 175 hours. Following exposure to chromium(VI), the ratio of blood chromium/lung chromium was 1.44 at 0.5 hours, 0.81 at 18 hours, 0.85 at 48 hours, and 0.96 at 168 hours after exposure. Chromium(III) particles of 1.5–1.8 μm had a single component pulmonary clearance curve with a half-life of 164 hours. Following exposure to chromium(III), the ratio of blood chromium/lung chromium was 0.39 at 0.5 hours, 0.24 at 18 hours, 0.22 at 48 hours, and 0.26 at 168 hours after exposure. Therefore, the amount of chromium(VI) transferred to the blood from the lungs was always at least 3 times greater than the amount of chromium(III) transferred (Suzuki et al. 1984). Other studies reporting absorption from the lungs are intratracheal injection studies (Baetjer et al. 1959b; Bragt and van Dura 1983; Visek et al. 1953; Wiegand et al. 1984, 1987). These studies indicate that 53–85% of chromium(VI) compounds (particle size <5 μm) are cleared from the lungs by absorption into the bloodstream or by mucociliary clearance in the pharynx; the rest remain in the lungs. Absorption by the bloodstream and mucociliary clearance was only 5–30% for chromium(III) compounds.

The kinetics of three chromium(VI) compounds, sodium chromate, zinc chromate, and lead chromate, were compared in rats in relation to their solubility. The rats received intratracheal injections of the ^{51}chromium-labeled compounds (0.38 mg chromium(VI)/kg as sodium chromate, 0.36 mg chromium(VI)/kg as zinc chromate, or 0.21 mg chromium(VI)/kg as lead chromate). Peak blood levels of ^{51}chromium were reached after 30 minutes for sodium chromate (0.35 μg chromium/mL), and 24 hours for zinc chromate (0.60 μg chromium/mL) and lead chromate (0.007 μg chromium/mL). At 30 minutes after administration, the lungs contained 36, 25, and 81% of the respective dose of the sodium, zinc, and lead chromate. On day 6, >80% of the dose of all three compounds had been cleared from the lungs, during which time, the disappearance from lungs followed linear first-order kinetics. The residual amounts left in the lungs on day 50 or 51 were 3.0, 3.9, and 13.9%, respectively. The results indicate that zinc chromate, which is ~1,000 times less soluble than sodium chromate, is more slowly absorbed from the lungs, but peak blood levels are higher than sodium chromate. Lead chromate was more poorly and slowly absorbed, as indicated by very low levels in blood and other tissues, and greater retention in the lungs (Bragt and van Dura 1983).

The fate of lead chromate(VI), chromium(VI) trioxide, chromium(III) oxide and chromium(III) sulfate were examined when solutions or suspensions of these chemicals were slowly infused into the tracheal lobe bronchus of sheep via bronchoscopic catheterization (Perrault et al. 1995). At 2, 3, 5, and 30 days, the samples of bronchoalveolar lavage were taken, and on day 31, the animals were sacrificed and lung specimens were examined for chromium particulates. There was no difference in lung particle concentrations among the four different compounds. The values ranged from $0.14x10^5$ to

$1.02x10^5$ particles/g dry tissue compared to control values of $0.03x10^5$. The alveolar clearance of slightly soluble chromium(III) oxide and chromium(III) sulfate was calculated to be 11 and 80 days, respectively. The insoluble lead chromate particles appeared to break up, forming isometric particles of lead chromate as well as lead-containing particulates that may have retarded clearance. Retention of chromium particulates from exposure to soluble chromium trioxide may have resulted in the formation of a less-soluble hydroxyl complex and/or chemical interaction between chromium and protein that prolongs the retention of the metal. Analyses of the particulates in lavage samples indicate that these diameters increase with time for lead chromate, decrease with time for chromium sulfate and chromium trioxide, and are unchanged for chromium(III) oxide. The authors state that their findings indicate that slightly soluble chromium(III) oxide and chromium sulfate that are chemically stable can be cleared from lungs at different rates, depending on the nature and morphology of the compound.

Amounts of total chromium were measured in lymphocytes, blood, and urine after intratracheal administration of either sodium dichromate(VI) or chromium(III) acetate hydroxide (a water-soluble chromium(III) compound) to male Wistar rats (Gao et al. 1993). The total amount of chromium administered was 0.44 mg chromium/kg body weight for each compound. The highest concentrations in tissues and urine occurred at 6 hours after treatment, the first time point examined. Mean chromium concentrations (n=4 rats per time point) from treatment with chromium(III) were 56.3 μg/L in whole blood, 96 μg/L in plasma, 0.44 $\mu g/10^{10}$ in lymphocytes, and 4,535.6 μg/g creatinine in urine. For treatment with chromium(VI) the levels were 233.2 μg/L for whole blood, 138 μg/L for plasma, 2.87 $\mu g/10^{10}$ for lymphocytes, and 2,947.9 μg/g creatinine in urine. The levels in lymphocytes in the chromium(III) treated animals were no different than in untreated animals. However, for chromium(VI) the lymphocyte levels were about 6-fold higher than control values. After 72 hours, the chromium levels were significantly reduced. These results suggest that absorbed chromium(III) compounds may be excreted more rapidly than absorbed chromium(VI) compounds because of a poorer ability to enter cells.

3.4.1.2 Oral Exposure

Chromium(III) is an essential nutrient required for normal energy metabolism. The Institute of Medicine (IOM 2001) of the NAS determined an adequate intake (e.g., a level typically consumed by healthy individuals) of 20–45 μg chromium(III)/day for adolescents and adults (IOM 2001). Currently, the biological target for the essential effects of chromium(III) is unknown. Chromodulin, also referred to as glucose tolerance factor (GTF), has been proposed as one possible candidate (Jacquamet et al. 2003). The function of chromodulin, an oligopeptide complex containing with four chromic ions, has not been

established; however, a possible mechanism is that chromodulin facilitates the interaction of insulin with its cellular receptor sites, although this has not been proven (Anderson 1998a, 2003; IOM 2001). Chromium(III) picolinate is a common form of chromium(III) nutritional supplementation.

Trivalent chromium is very poorly absorbed from the gastrointestinal tract. Typically, ≤1% of an orally administered dose of trivalent chromium has been recovered in the urine of experimental animals of humans (Aitio et al. 1984; Anderson et al. 1983; Doisy et al. 1971; Donaldson and Barreras 1966; Gargas et al. 1994; Garcia et al. 2001; Kerger et al. 1996a) or experimental animals (Donaldson and Barreras 1966; Febel et al. 2001). Oral absorption of trivalent chromium complexed with an organic ligand is similarly low and not higher than inorganic forms **(**Anderson et al. 1996; Gonzalez-Vergara et al. 1981). Bypassing the stomach by infusing trivalent chromium into the duodenum or jejunum resulted in at most 1–2% of the dose being absorbed in humans (Donaldson and Barreras 1966), or 1% (Febel et al. 2001) to 4% in the rat (Donaldson and Barreras 1966).

Approximately 0.5–2.0% of dietary chromium(III) is absorbed via the gastrointestinal tract of humans (Anderson 1986; Anderson et al. 1983) as inferred from urinary excretion measurements. The absorption fraction is dependent on the dietary intake. At low levels of dietary intake (10 μg), ~2.0% of the chromium was absorbed. When intake was increased by supplementation to ≥40 μg, the absorption decreased to ~0.5% (Anderson 1986; Anderson et al. 1983). Net absorption of chromium(III) by a group of 23 elderly subjects who received an average of 24.5 μg/day (0.00035 mg chromium(III)/kg/day) from their normal diets was calculated to be 0.6 μg chromium(III)/day, based on an excretion of 0.4 μg chromium/day in the urine and 23.9 μg chromium/day in the feces, with a net retention of 0.2 μg/day. Thus, about 2.4% was absorbed. The retention was considered adequate for their requirements (Bunker et al. 1984).

The absorption fraction of soluble chromium(III), as chromium picolinate, is greater than $CrCl_3$ (DiSilvestro and Dy 2007; Gargas et al. 1994). Following ingestion of 400 μg chromium(III)/day as chromium picolinate (in a capsule) for 3 consecutive days, mean absorption fraction in eight healthy adults was 2.8% (±1.4 % standard deviation [SD]; Gargas et al. 1994). Based on urinary excretion following oral administration of a single dose (200 μg chromium(III)) of four different chromium(III) supplements to healthy women (n=24; cross-over design), the absorption of chromium picolinate was higher than that of chromium chloride, chromium polynicotinate, and chromium nicotinate-glucinate; estimates of oral absorption were not reported (DiSilvestro and Dy 2007). Urinary excretion of chromium following administration of chromium picolinate was approximately 16-fold higher than that following

administration of chromium chloride and approximately 2-fold greater than that following administration of the two nicotinate complexes.

Association of chromium with chelating agents, which may be naturally present in feed, can alter the bioavailability from food. In rats that were given ^{51}Cr-chromium(III) trichloride mixed with chelating agents, either oxalate or phytate, phytate significantly ($p<0.05$) decreased the levels of radioactivity in blood, whole body, and urine achieved with chromium(III) trichloride alone (Chen et al. 1973). Oxalate, however, greatly increased the levels in blood, whole body, and urine. The oxalate served as a strong ligand to protect against the tendency of chromium(III) to form insoluble macromolecular chromium oxides at physiological pH. Fasted rats absorbed significantly more ^{51}chromium than did nonfasted rats, indicating that the presence of food in the gastrointestinal tract slows the absorption of chromium. Results of an *in vitro* experiment in this study indicated that the midsection had greater uptake than the duodenum or ileum and that oxalate significantly ($p<0.05$) increased, while phytate significantly ($p<0.05$) decreased the transport of chromium(III) across all three sections, paralleling the *in vivo* results. Ethylenediamine tetraacetic acid (EDTA) and citrate were also tested in the *in vitro* system, but were found to have no effect on chromium(III) intestinal transport; therefore, these chelating agents were not tested *in vivo* (Chen et al. 1973).

The absorption fraction of soluble chromium(VI) is higher than that of soluble chromium(III) (Anderson et al. 1983; Donaldson and Barreras 1966; Kerger et al. 1996a). Average absorption fractions, determined from cumulative urinary excretion in 8 healthy adults who ingested 5 mg chromium (in 10 mg Cr/L drinking water) as $CrCl_3$ or $K_2Cr_2O_7$ were 0.13% (±0.04, standard error [SE]) and 6.9% (±3.7, SE), respectively. Chromium(VI) can be reduced to chromium(III) when placed in an ascorbic acid solution (Kerger et al. 1996a). When $K_2Cr_2O_7$ was ingested in orange juice (where it was reduced and may have formed complexes with constituents of the juice), the mean absorption fraction was 0.60% (±0.11, SE; Kerger et al. 1996a). Plasma concentrations generally peaked around 90 minutes following exposure for all three chromium mixtures tested. Based on measurements of urinary excretion of chromium in 15 female and 27 male subjects who ingested 200 µg chromium(III) as $CrCl_3$, the absorption fraction was estimated to be approximately 0.4% (Anderson et al. 1983). The absorption fraction of chromium(VI) (as sodium chromate) was substantially higher when administered directly into the duodenum (approximately 10%) compared to when it is ingested (approximately 1.2%), whereas the absorption fraction for $CrCl_3$ was similar when administered into the small intestine (0.5%; Donaldson and Barreras 1966). These results are consistent with studies that have shown that gastric juice can reduce chromium(VI) to chromium(III) (De Flora et al. 1987a).

The absorption of chromium(VI) and chromium(III) was measured in four male and two female volunteers (ages ranging from 25 to 39 years) treated orally with potassium chromate (chromium(VI)) or chromic oxide (chromium(III)) in capsules at doses of 0.005 and 1.0 mg/kg/day, respectively (Finley et al. 1996b). Subjects were exposed to each compound for 3 days. Based on urinary excretion data, the mean absorption of potassium chromate was 3.4% (range 0.69–11.9%). No statistically significant increase in urinary chromium was observed during chromic oxide dosing, indicating that little, if any, was absorbed. In a follow-up study by the same group (Finley et al. 1997), five male volunteers ingested a liter, in three volumes of 333 mL, of deionized water containing chromium(VI) concentrations ranging from 0.1 to 10.0 mg/L (approximately 0.001–0.1 mg chromium(VI)/kg/day) for 3 days. A dose-related increase in urinary chromium was seen in all subjects and the percent of the dose excreted ranged from <2 to 8%. Dose-related increases in plasma and erythrocyte chromium levels were also observed.

In a repeated dose study, three healthy adults ingested chromium(VI) (as $K_2Cr_2O_7$) in water at 5 mg chromium/day for 3 consecutive days (Kerger et al. 1997). Three divided doses were taken at approximately 6-hour intervals over a 5–15-minute period. After at least 2 days without dosing, the 3-day exposure regimen was repeated at 10 mg chromium/day. Estimated doses based on body weight were 0.05 and 0.1 mg/kg/day, respectively. Bioavailability based on 4-day urinary excretion was 1.7% (range 0.5–2.7%) at 0.05 mg chromium(VI)/kg/day and 3.4% (range 0.8–8.0%) at 0.1 mg chromium(VI)/kg/day. Absorption of 0.05 mg chromium(VI)/kg appeared to be somewhat lower when given as three divided doses rather than when given as a single bolus dose (1.7 versus 5.7%).

Uptake of potassium dichromate was determined in a man who was given 0.8 mg of chromium(VI) in drinking water 5 times each day for 17 days (Paustenbach et al. 1996). Steady-state concentrations of chromium in blood were attained after 7 days. Red blood cell and plasma levels returned to background levels within a few days after exposure was stopped. The data are consistent with a bioavailability of 2% and a plasma elimination half-life of 36 hours.

Studies with ^{51}chromium in animals indicate that chromium and its compounds are also poorly absorbed from the gastrointestinal tract after oral exposure. When radioactive sodium chromate (chromium(VI)) was given orally to rats, the amount of chromium in the feces was greater than that found when sodium chromate was injected directly into the jejunum. Since chromium(III) is absorbed less readily than chromium(VI) by the gastrointestinal tract, these results are consistent with evidence that the gastric environment has a capacity to reduce chromium(VI) to chromium(III). Furthermore, the administration

of radioactive chromium(III) or chromium(VI) compounds directly into the jejunum decreased the amount of chromium recovery in the feces indicating that the jejunum is the absorption site for chromium (Donaldson and Barreras 1966). Absorption of either valence state was ≤1.4% of the administered oral dose in rats (Sayato et al. 1980) and hamsters (Henderson et al. 1979). Based on distribution (see Section 3.3.2.2) and excretion (see Section 3.3.4.2) studies in rats administered chromium by gavage for 2–14 days from various sources, that is, from sodium chromate (chromium(VI)), from calcium chromate (chromium(VI)), or from soil contaminated with chromium (30% chromium(VI) and 70% chromium(III)), the low gastrointestinal absorption of chromium from any source was confirmed. Chromium appeared to be better absorbed from the soil than from chromate salts, but <50% of the administered chromium could be accounted for in these studies, partly because not all tissues were examined for chromium content and excretion was not followed to completion (Witmer et al. 1989, 1991). Adult and immature rats given chromium(III) chloride absorbed 0.1 and 1.2% of the oral dose, respectively (Sullivan et al. 1984). This suggests that immature rats may be more susceptible to potential toxic effects of chromium(III) compounds.

Treatment of rats by gavage with a nonencapsulated lead chromate pigment or with a silica-encapsulated lead chromate pigment resulted in no measurable blood levels of chromium (detection limit=10 μg/L) after 2 or 4 weeks of treatment or after a 2-week recovery period. However, kidney levels of chromium were significantly higher in the rats that received the nonencapsulated pigment than in the rats that received the encapsulated pigment, indicating that silica encapsulation reduces the gastrointestinal bioavailability of chromium from lead chromate pigments (Clapp et al. 1991).

The issue of whether or not chromium(VI) absorption occurs only when or principally when the reducing capacity of the gastrointestinal tract is exhausted is a factor to consider in evaluating and interpreting oral dosing bioassays in animals and human epidemiology studies of health outcomes related to ingestion exposures to chromium. Potentially, tumor responses could be enhanced if the reducing capacities of saliva and stomach fluid were exhausted. This is more likely to occur at the relatively high doses of chromium(VI) administered in animal bioassays than at doses experienced by humans from environmental exposures. However, results of experimental studies of chromium absorption in humans have not found evidence for an effect of limited of reducing capacity on absorption of chromium. The range of doses of chromium administered to humans in these different studies was considerable and demonstrated oral bioavailability at all doses. Donaldson and Barreras (1996) administered 20 ng of radiolabeled chromium(VI), Kerger et al. (1996a) administered 5 mg of chromium(VI), Finley et al. (1996b) administered 0.005 mg/kg-day of chromium(VI) for 3 days, and Finley et al. (1997) administered

0.1, 0.5, 1.0, 5.0 or 10 mg/day of chromium(VI) for 4 days. In the Finley et al. (1997) study, the percent of the administered dose of chromium(VI) recovered in the urine did not increase with dose. The results of these studies do not indicate that oral absorption of administered chromium(VI) only begins to occur when the reducing capacity of the stomach is exhausted, and are consistent with estimates of gastrointestinal reducing capacity (De Flora 2000; Proctor et al. 2002).

3.4.1.3 Dermal Exposure

Both chromium(III) and chromium(VI) can penetrate human skin to some extent, especially if the skin is damaged. Systemic toxicity has been observed in humans following dermal exposure to chromium compounds, indicating significant cutaneous absorption (see Section 3.2.3). Fourteen days after a salve containing potassium chromate was applied to the skin of an individual to treat scabies, appreciable amounts of chromium were found in the blood, urine, feces, and stomach contents (Brieger 1920) (see Section 3.4.2.3). It should be noted that the preexisting condition of scabies or the necrosis caused by the potassium chromate (see Section 3.2.3) could have facilitated dermal absorption of potassium chromate. Potassium dichromate (chromium(VI)), but not chromium(III) sulfate, penetrated the excised intact epidermis of humans (Mali et al. 1963). Dermal absorption by humans of chromium(III) sulfate in aqueous solution was negligible, with slightly larger amounts of chromium(III) nitrate in aqueous solution absorbed. The absorption of chromium(III) chloride was similar to potassium dichromate(VI) (Samitz and Shrager 1966). Chromium(III) from a concentrated chromium sulfate solution at pH 3 penetrated cadaverous human skin at a rate of $5x10^{-11}$ cm/sec, compared with a rate for chromium(VI) (source unspecified) of $5x10^{-7}$ cm/second (Spruit and van Neer 1966). In contrast, both chromium(VI) from sodium chromate and chromium(III) from chromium trichloride penetrated excised human mammary skin at similar rates, but the rate was generally slightly faster for chromium(VI). Absolute rates of absorption in nmol chromium/hour/cm^2 increased with increasing concentration of both chromium(VI) and chromium(III) (Wahlberg 1970). The average rate of systemic uptake of chromium in four volunteers submersed up to the shoulders in a tub of chlorinated water containing a 22 mg chromium(VI)/L solution of potassium dichromate for 3 hours was measured to be $1.5x10^{-4}$ μg/cm^2-hour based on urinary excretion of total chromium (Corbett et al. 1997).

The influence of solvent on the cutaneous penetration of potassium dichromate by humans has been studied. The test solutions of potassium dichromate in petrolatum or in water were applied as occluded circular patches of filter paper to the skin. Results with dichromate in water revealed that chromium(VI) penetrated beyond the dermis and penetration reached steady state with resorption by the lymph and

blood vessels by 5 hours. About 10 times more chromium penetrated when potassium dichromate was applied in petrolatum than when applied in water. About 5 times more chromium penetrated when potassium dichromate was applied than when a chromium trichloride glycine complex was applied (Liden and Lundberg 1979). The rates of absorption of solutions of sodium chromate from the occluded forearm skin of volunteers increased with increasing concentration. The rates were 1.1 μg chromium(VI)/ cm^2/hour for a 0.01 M solution, 6.4 μg chromium(VI)/cm^2/hour for a 0.1 M solution, and 10 μg chromium(VI)/cm^2/hour for a 0.2 M solution (Baranowska-Dutkiewicz 1981).

Chromium and its compounds are also absorbed dermally by animals. The dermal absorption of sodium chromate (chromium(VI)) by guinea pigs was somewhat higher than that of chromium(III) trichloride, but the difference was not significant. At higher concentrations (0.261–0.398 M), absorption of sodium chromate was statistically higher than that of chromium trichloride. The peak rates of absorption were 690–725 and 315–330 nmol/hour/cm^2 for sodium chromate at 0.261–0.398 M and chromium trichloride at 0.239–0.261 M, respectively. Percutaneous absorption of sodium chromate was higher at pH≥6.5 compared with pH≤5.6 (Wahlberg and Skog 1965).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Examination of tissues from Japanese chrome platers and chromate refining workers at autopsy revealed higher chromium levels in the hilar lymph node, lung, spleen, liver, kidney, and heart, compared to normal healthy males (Teraoka 1981). Analysis of the chromium concentrations in organs and tissues at autopsy of a man who died of lung cancer 10 years after his retirement from working in a chromate producing plant for 30 years revealed measurable levels in the brain, pharyngeal wall, lung, liver, aorta, kidney, abdominal rectal muscle, suprarenal gland, sternal bone marrow, and abdominal skin. The levels were significantly higher than in five controls with no occupational exposure to chromium. The man had been exposed mainly to chromium(VI), with lesser exposure to chromium(III) as the chromite ore (Hyodo et al. 1980). The levels of chromium were higher in the lungs, but not in the liver or kidneys, of autopsy specimens from 21 smeltery and refinery workers in North Sweden compared with that for a control group of 8 individuals. The amount of enrichment in the lungs decreased as the number of elapsed years between retirement and death increased (Brune et al. 1980). Tissues from three individuals having lung cancer who were industrially exposed to chromium were examined by Mancuso (1997b). One was employed for 15 years as a welder, a second worked for 10.2 years, and a third for 31.8 years in ore milling and preparations and boiler operations. The three cumulative chromium exposures for the three

workers were 3.45, 4.59, and 11.38 mg/m^3 years, respectively. Tissues from the first worker were analyzed 3.5 years after last exposure, the second worker 18 years after, and the third worker 0.6 years after last exposure. All tissues from the three workers had elevated levels of chromium with the possible exception of neural tissues. Levels were orders of magnitude higher in lungs than other tissues. The highest lung level reported was 456 mg/10 g tissue in the first worker, 178 in the second worker, and 1,920 for the third worker. There were significant chromium levels in the tissue of the second worker even though he had not been exposed to chromium for 18 years. Chromium concentrations in lung tissues from autopsy samples were 5 times higher in subjects who originated from the Ruhr and Dortmund regions of Germany, where emissions of chromium are high, than in subjects from Munster and vicinity. The lung concentrations of chromium increased with increasing age. Men had twice as high concentrations of chromium in the lungs than did women, which may reflect the greater potential for occupational exposure by men, the higher vital capacity of men, and possibly a greater history of smoking (Kollmeier et al. 1990).

Chromium may be transferred to fetuses through the placenta and to infants via breast milk. Analysis of chromium levels in women employees of a dichromate manufacturing facility in Russia during and after pregnancy revealed that the exposed women had significantly higher levels of chromium in blood and urine during pregnancy, in umbilical cord blood, placentae, and breast milk at child birth, and in fetuses aborted at 12 weeks than did nonexposed controls (Shmitova 1980). The reliability of this study is suspect because the levels of chromium reported in the blood and urine of the control women were much higher than usual background levels of chromium in these biological fluids (see Section 6.5), perhaps due to problems with analytical methods. Measurement of the chromium content in 255 samples from 45 lactating American women revealed that most samples contained <0.4 μg/L, and the mean value was 0.3 μg/L (Casey and Hambidge 1984). While these probably represent background levels in women whose main exposure to chromium is via the diet, the findings indicate that chromium may be transferred to infants via breast milk.

The distribution of radioactivity in rats given ^{51}chromium as sodium dichromate intratracheally was followed for 40 days by autoradiography and scintillation counting. Three days after the administration of 0.01 mg chromium(VI)/m^3 as radioactive sodium dichromate, the tissue distribution based on the relative concentrations in the tissue was lung > kidney > gastrointestinal tract > erythrocytes > liver > serum > testis > skin. Twenty-five days after dosing, the tissue distribution was lung > kidney > erythrocytes > testis > liver > serum > skin > gastrointestinal tract. Kidney, erythrocytes, and testis maintained their chromium levels for a period of 10–15 days before decreasing (Weber 1983). The

distribution of chromium(VI) compared with chromium(III) was investigated in guinea pigs after intratracheal instillation of potassium dichromate or chromium trichloride. At 24 hours after instillation, 11% of the original dose of chromium from potassium dichromate remained in the lungs, 8% in the erythrocytes, 1% in plasma, 3% in the kidney, and 4% in the liver. The muscle, skin, and adrenal glands contained only a trace. All tissue concentrations of chromium declined to low or nondetectable levels in 140 days with the exception of the lungs and spleen. After chromium trichloride instillation, 69% of the dose remained in the lungs at 20 minutes, while only 4% was found in the blood and other tissues, with the remaining 27% cleared from the lungs and swallowed. The only tissue that contained a significant amount of chromium 2 days after instillation of chromium trichloride was the spleen. After 30 and 60 days, 30 and 12%, respectively, of the chromium(III) was retained in the lungs, while only 2.6 and 1.6%, respectively, of the chromium(VI) dose was retained in the lung (Baetjer et al. 1959a).

3.4.2.2 Oral Exposure

Autopsy studies in the United States indicate that chromium concentrations in the body are highest in kidney, liver, lung, aorta, heart, pancreas, and spleen at birth and tend to decrease with age. The levels in liver and kidney declined after the second decade of life. The aorta, heart, and spleen levels declined rapidly between the first 45 days of life and 10 years, with low levels persisting throughout life. The level in the lung declined early, but increased again from mid life to old age (Schroeder et al. 1962).

The distribution of chromium in human body tissue after acute oral exposure was determined in the case of a 14-year-old boy who ingested 7.5 mg chromium(VI)/kg as potassium dichromate. Despite extensive treatment by dialysis and the use of the chelating agent British antilewisite, the boy died 8 days after admission to the hospital. Upon autopsy, the chromium concentrations were as follows: liver, 2.94 mg/100 cc (normal, 0.016 mg/100 cc); kidneys, 0.64 and 0.82 mg/100 cc (normal, 0.06 mg/100 cc); and brain, 0.06 mg/100 cc (normal, 0.002 mg/100 cc) (Kaufman et al. 1970). Although these data were obtained after extensive treatment to rid the body of excess chromium, the levels of chromium remaining after the treatment clearly demonstrate that these tissues absorbed at least these concentrations after an acute, lethal ingestion of a chromium(VI) compound.

Chromium may be transferred to infants via breast milk as indicated by breast milk levels of chromium in women exposed occupationally (Shmitova 1980) or via normal levels in the diet (Casey and Hambidge 1984). It has been demonstrated that in healthy women, the levels of chromium measured in breast milk are independent of serum chromium levels, urinary chromium excretion, or dietary intake of chromium

(Anderson et al. 1993, Mohamedshah et al. 1998), but others (Engelhardt et al. 1990) have disputed this observation.

The tissue distribution of chromium was studied in rats administered chromium from a variety of sources. In one experiment, sodium chromate in water was administered by gavage for 7 days at 0, 1.2, 2.3, or 5.8 mg chromium(VI)/kg/day. Very little chromium (generally <0.5 μg/organ) was found in the organs analyzed (liver, spleen, lung, kidney, and blood) after administration of the two lower doses. The levels were generally comparable to those in controls. After 5.8 mg/kg/day, the largest amount of chromium (expressed as μg chromium/whole organ) was found in the liver (≈22 μg), followed by the kidney (≈7.5 μg), lung (≈4.5 μg), blood (≈2 μg), and spleen (≈1 μg). The total amount of chromium in these tissues represented only 1.7% of the final dose of 5.8 mg/kg/day, but not all organs were analyzed. In the next experiment, rats were exposed by gavage to 7 mg chromium/kg/day for 7 days from various sources: (1) sodium chromate; (2) calcium chromate; (3) soil containing chromium (30% chromium(VI), 70% chromium(III)); or (4) a mixture of calcium chromate and the contaminated soil. The highest levels of chromium were found in liver, spleen, kidney, lung, blood, brain, and testes after dosing with sodium chromate, but the relative levels in these tissues after the other treatments followed no consistent pattern. Rats gavaged for 14 days with 13.9 mg chromium/kg/day from the four different sources had higher levels of chromium in the tissues after they were dosed with the contaminated soil or the mixture of calcium chromate and the contaminated soil than with either of the chromate salts alone. Thus, the relative organ distribution of chromium depends on the source of chromium (Witmer et al. 1989, 1991). Components in soil may affect the oxidation state and the binding of chromium to soil components, and pH of the soil may also affect the bioavailability from soil.

The chromium content in major organs (heart, lung, kidney, liver, spleen, testes) of mice receiving drinking water that provided doses of 4.8, 6.1, or 12.3 mg chromium(III)/kg/day as chromium trichloride or 4.4, 5.0, or 14.2 mg chromium(VI)/kg/day as potassium dichromate was determined after 1 year of exposure. Chromium was detected only in the liver in the chromium(III)-treated mice. Mice treated with chromium(VI) compounds had accumulation in all of the above organs, with the highest levels reported in the liver and spleen. Liver accumulation of chromium was 40–90 times higher in the chromium(VI)-treated group than in the chromium(III)-treated group (Maruyama 1982). Chromium levels in tissue (bone, kidney liver, spleen) were 9 times higher in rats given chromium(VI) as potassium chromate in drinking water for 1 year than in rats given the same concentration of chromium(III) as chromium trichloride (MacKenzie et al. 1958). In rats exposed to potassium chromate in the drinking water for 3 or 6 weeks, a general trend of increasing chromium concentration with time of exposure was apparent in the

liver and kidneys, but only the kidneys showed a difference in the concentration after exposure to 100 and 200 ppm. Blood concentrations were almost saturated by 3 weeks with little further accumulation by 6 weeks. No chromium was detected in the lungs after drinking water exposure (Coogan et al. 1991a). After acute oral dosing with radiolabeled chromium trichloride (1 μCi for immature rats, 10 μCi for adults), adult and neonatal rats accumulated higher levels of chromium in the kidneys than in the liver. At 7 days after dosing, the liver and kidney contained 0.05 and 0.12% of the dose, respectively, in the neonates and 0.002 and 0.003% of the dose, respectively, in the adult rats. The carcass contained 0.95% of the dose in the neonates and 0.07% of the dose in adult rats. The lungs contained 0.0088% of the dose in neonates and 0.0003% of the dose in adult rats. No chromium(III) was detected in the skeleton or muscle. Approximately 35 and 0.2% of the administered dose of chromium(III) at day 7 was retained in the gut of neonates and adults, respectively (Sullivan et al. 1984).

The distribution of potassium chromate(VI) was compared in male Fisher rats and C57BL/6J mice exposed either by drinking water (8 mg chromium(VI)/kg/day for 4 and 8 weeks) or by intraperitoneal injection (0.3 and 0.8 mg chromium(VI)/kg/day for 4 or 14 days) (Kargacin et al. 1993). The concentrations of chromium (μg/g wet tissue) after drinking water exposures for 8 weeks in mice were: liver 13.83, kidney 4.72, spleen 10.09, femur 12.55, lung 1.08, heart 1.02, muscle 0.60, and blood 0.42. These concentrations were not markedly different than for 4-week exposures. For rats, the concentrations were: liver 3.59, kidney 9.49, spleen 4.38, femur 1.78, lung 0.67, heart 1.05, muscle 0.17, and blood 0.58. These results demonstrate that considerable species differences exist between mice and rats and need to be factored into any toxicological extrapolations across species even if the routes of administration are the same. In the drinking water experiments, blood levels in rats and mice were comparable, but in intraperitoneal injection experiments, rats' levels were about 8-fold higher than mice after 4 days of exposure. This difference appeared to be due to increased sequestering by rat red blood cells, since accumulation in white blood cells was lower in rats than mice. The higher incidence of red cell binding was also associated with greater binding of chromium to rat hemoglobin.

The feeding of five male Wistar rats at 0.49 mg chromium(III)/kg/day as chromium(III) chloride for 10 weeks resulted in increased chromium levels in liver, kidney, spleen, hair, heart, and red blood cells (Aguilar et al. 1997). Increases were highest in kidney (0.33 μg/g wet tissue in controls versus 0.83 μg/g in treated animals) and erythrocytes (1.44 μg/g wet tissue in controls versus 3.16 μg/g in treated animals).

The higher tissue levels of chromium after administration of chromium(VI) than after administration of chromium(III) (MacKenzie et al. 1958; Maruyama 1982; Witmer et al. 1989, 1991) reflect the greater

tendency of chromium(VI) to traverse plasma membranes and bind to intracellular proteins in the various tissues, which may explain the greater degree of toxicity associated with chromium(VI). In an experiment to determine the distribution of chromium in red and white blood cells, rats were exposed orally to 0.0031 mg/kg of ^{51}chromium(VI) as sodium chromate. The ^{51}chromium content of the fractionated blood cells was determined either 24 hours or 7 days after exposure. After 24 hours, the white blood cells contained much more ^{51}chromium (≈250 pg chromium/billion cells) than did the red blood cells (≈30 pg chromium/billion cells). After 7 days, the ^{51}chromium content of the white blood cells was reduced only 2.5-fold, while that of the red blood cells was reduced 10-fold. Thus, white blood cells preferentially accumulated chromium(VI) and retained the chromium longer than did the red blood cells. As discussed in Section 3.4.2.4, a small amount of chromium(III) entered red blood cells of rats after intravenous injection of ^{51}chromium trichloride, but no ^{51}chromium was detectable in white blood cells (Coogan et al. 1991b).

Twelve pregnant female albino rats (Druckrey strain) and 13 Swiss albino mice were exposed to 500 ppm potassium dichromate(VI) in their drinking water during pregnancy up to 1 day before delivery (Saxena et al. 1990a). The chromium(VI) daily intake was calculated to be 11.9 mg chromium(VI)/day for the rats and 3.6 mg chromium(VI)/day for mice which were considered to be maximal nontoxic doses for both species. In rats, concentrations of chromium were 0.067, 0.219, and 0.142 μg/g fresh weight in maternal blood, placenta, and fetuses, respectively, and 0.064, 0.304, and 0.366 μg/g fresh weight in mice, respectively. In treated rats, chromium levels were 3.2-fold higher in maternal blood, 3-fold higher in placenta, and 3.1-fold higher in fetal tissue when compared to control values. In treated mice, chromium levels were 2.5-fold higher in maternal blood, 3.2-fold higher in placenta, and 9.6-fold higher in fetuses when compared to control values. In treated mice, there was a significant elevation in chromium levels in placental and fetal tissues over maternal blood levels, and a significant increase in chromium levels in fetal tissue over placental concentrations when compared to controls. These differences were not observed in rats, indicating that the distribution patterns in mice and rats are different.

A study of transplacental transfer of chromium(III) in different forms indicated that placental transport varies with the chemical form. Male and female rats were fed either a commercial diet that contained 500 ppb chromium or a 30% Torula yeast diet that contained <100 ppb chromium. They were also given drinking water with or without 2 ppm chromium(III) added as chromium acetate monohydrate. The rats were mated and immediately after delivery, the neonates were analyzed for chromium content. The neonates whose dams were fed the commercial diet contained almost twice as much chromium as those whose dams were fed the chromium-deficient yeast diet. Addition of chromium(III) acetate to the

drinking water of the yeast-fed rats (2 ppm) did not increase the levels of chromium in the neonates. Administration of chromium(III) trichloride intravenously or by gavage before mating, during mating, or during gestation resulted in no or only a small amount of chromium in the neonates. Administration of chromium(III) in the form of GTF from Brewer's yeast by gavage during gestation resulted in chromium levels in the litters that were 20–50% of the dams' levels. The results indicate that fetal chromium is derived from specific chromium complexes in the diet (e.g., GTF) (Mertz et al. 1969).

3.4.2.3 Dermal Exposure

The findings of toxic effects in the heart, stomach, blood, muscles, and kidneys of humans who were dermally exposed to chromium compounds is suggestive of distribution to these organs (see Section 3.2.3.2). Fourteen days after a salve containing potassium chromate(VI) was applied to the skin of an individual to treat scabies, appreciable amounts of chromium were found in the blood (2–5 mg/100 mL), urine (8 mg/L), feces (0.61 mg/100 g), and stomach contents (0.63 mg/100 mL) (Brieger 1920). The preexisting condition of scabies or the necrosis caused by the potassium chromate could have facilitated its absorption. A transient increase in the levels of total chromium in erythrocytes and plasma was observed in subjects immersed in a tank of chlorinated water containing potassium dichromate(VI) (Corbett et al. 1997).

Chromium compounds are absorbed after dermal administration by guinea pigs. Measurement of ^{51}chromium in the organs and body fluids revealed distribution, due to dermal absorption of chromium(III) and chromium(VI) compounds, to the blood, spleen, bone marrow, lymph glands, urine, and kidneys. Absorption was greater for chromium(VI) than for chromium(III) (see Section 3.4.1.3) (Wahlberg and Skog 1965).

3.4.2.4 Other Routes of Exposure

The distribution of chromium(III) in humans was analyzed using a whole-body scintillation scanner, whole-body counter, and plasma counting. Six individuals given an intravenous injection of ^{51}chromium(III) as chromium trichloride had >50% of the blood plasma chromium(III) distributed to various body organs within hours of administration. The liver and spleen contained the highest levels. After 3 months, the liver contained half of the total body burden of chromium. The study results indicated a three-compartment model for whole-body accumulation and clearance of chromium(III). The half-lives were 0.5–12 hours for the fast component, 1–14 days for the medium component, and 3–12 months for the slow component (Lim et al. 1983).

An *in vitro* study in human blood showed that chromium(VI) was rapidly cleared from the plasma (Corbett et al. 1998). The reduction capacity appears to be concentration dependent and is overwhelmed at spike concentrations between 2,000 and 10,000 µg/L. High chromium(VI) concentrations (10,000 µg/L spike concentration) resulted in an accumulation of chromium(VI) in the erythrocytes and a lower plasma:erythrocyte ratio of total chromium. This study also found that the plasma reduction capacity was enhanced by a recent meal.

Both human and rat white blood cells accumulated more ^{51}chromium per cell than red blood cells after *in vitro* exposure of whole blood to ^{51}chromium(VI). The uptake of chromium by rat blood cells was also measured after intravenous exposure to ^{51}chromium(VI) as sodium chromate. After intravenous exposure, the white blood cells contained significantly more ^{51}chromium (≈30 pg chromium/billion cells) than the red blood cells (≈4 pg chromium/billion cells), and the amount of ^{51}chromium in the cells was the same after 24 hours as it was after 1 hour. The amount of ^{51}chromium in the white blood cells, but not in the red blood cells, decreased by approximately 1.7-fold after 7 days. When rats were injected intravenously with 20 ng of radiolabeled sodium chromate (chromium(VI)) or radiolabeled chromium trichloride (chromium(III)), the amount of chromium was ≈2 pg/billion red blood cells but not detectable in white blood cells after injection of chromium(III) chloride. The amount of chromium was ≈5 pg/billion red blood cells and ≈60 pg/billion white blood cells after injection of sodium chromate (Coogan et al. 1991b).

The distribution pattern in rats treated with sodium chromite (chromium(III)) by intravenous injection revealed that most of the chromium was concentrated in the reticuloendothelial system, which, together with the liver, accumulated 90% of the dose. The accumulation in the reticuloendothelial system was thought to result from colloid formation by chromite at physiological pH. Organs with detectable chromium levels 42 days postinjection were: spleen > liver > bone marrow > tibia > epiphysis > lung > kidney. Chromium trichloride given to rats by intravenous injection also concentrated in the liver, spleen, and bone marrow (Visek et al. 1953). In rats administered chromium(III) nitrate intraperitoneally for 30 or 60 days, the highest levels of chromium were found in the liver, followed by the kidneys, testes, and brain. The levels increased with increased doses but not linearly. The levels in the kidneys, but not the other organs, increased significantly with duration (Tandon et al. 1979).

Whole-body analysis of mice given a single intraperitoneal injection of 3.25 mg chromium(III)/kg as chromium trichloride showed that chromium trichloride was released very slowly over 21 days: 87% was

retained 3 days after treatment, 73% after 7 days treatment, and 45% after 21 days. In contrast, mice given a single intraperitoneal injection of 3.23 chromium(VI)/kg as potassium dichromate retained only 31% of the chromium(VI) dose at 3 days, 16% at 7 days and 7.5% at 21 days. Mice injected weekly with chromium(III) compounds at 17% of the LD_{50} retained 6 times the amount of chromium as mice injected with chromium(VI) compounds at 17% of the LD_{50}. The retention of chromium(III) was attributed to its ability to form coordination complexes with tissue components such a proteins and amino acids (Bryson and Goodall 1983).

In rats injected intraperitoneally with 2 mg chromium(VI)/kg/day as potassium chromate 6 days/week for 45 days, the mean levels of chromium (μg chromium/g wet weight) were 25.68 in the liver, 40.61 in the kidney, 7.56 in the heart, and 4.18 in the brain (Behari and Tandon 1980).

In rats injected subcutaneously with 5.25 mg chromium(VI)/kg as potassium dichromate, most of the chromium in the tissues analyzed was found in the red blood cells with a peak level (63 μg chromium/g) achieved 24 hours after dosing. White blood cells were not analyzed for chromium content. Whole plasma contained 2.7–35 μg/mL and the plasma ultrafiltrate contained 0.15–0.79 μg/mL. Tissue distribution 48 hours after dosing was as follows: 221.2 μg/g in renal cortex, 110.0 μg/g in liver, 103.0 μg/g in spleen, 86.8 μg/g in lung, 58.9 μg/g in renal medulla, and 8.8 μg/g in bone, compared with 2.28–5.98 μg/g in any tissues in controls. When rats were given repeated subcutaneous injections of 1.05 mg chromium(VI)/kg/day, every other day for 2, 4, 8, 10, or 12 weeks, most of the chromium was again found in the red blood cells. However, while red blood cell levels rose progressively during treatment, levels as high as those seen after a single dose were never achieved, even when the total dose exceeded the dose in the single injection experiment 10-fold. The tissue levels of chromium determined 48 hours after the last dose in the rats injected for 12 weeks were of the same order of magnitude as those seen after a single injection. These results suggest little tendency of soluble chromium(VI) compounds to accumulate in tissues with repeated exposure (Mutti et al. 1979).

In an *in vitro* study, whole blood samples were spiked with water-soluble chromium(VI) or chromium(III) compounds. The results showed a greater level of chromium inside erythrocytes after treatment with chromium(VI) compounds, compared to chromium(III) compounds. The investigators reported that only chromium(VI) compounds are taken up by erythrocytes and, presumably after reduction to chromium(III), form complexes with intracellular proteins that could not be eliminated (Lewalter et al. 1985).

The distribution of radioactivity was compared in mouse dams and fetuses following the intravenous injection of the dams with ^{51}Chromium labelled-sodium dichromate(VI) or ^{51}chromium labelled-chromium(III) trichloride. In the maternal tissues, the highest levels of radioactivity from both treatments were achieved in the renal cortex, but the concentration of radioactivity in the tissues of dams given the hexavalent form was much higher than that of the dams given the trivalent form. The patterns of distribution of radioactivity in other tissues were identical regardless of administered valence state, with the skeleton, liver, kidneys, and ovaries accumulating the highest levels and the brain and muscle accumulating the lowest levels. The serum concentration of radioactivity after treatment with chromium(III) was 3 times higher than that after treatment with chromium(VI). Radioactivity after treatment with both valence forms crossed the placenta, but the radioactivity from the hexavalent form crossed more readily. For chromium(VI), ≈12% of the maternal serum concentration of radioactivity was found in the fetuses when the dams were administered sodium dichromate in mid-gestation (days 12–15). When the dams were injected in late gestation (days 16–18), ≈19% of the radioactivity in maternal serum was found in the fetuses. For chromium(III), the fetal concentration of radioactivity was only ≈0.4% of the maternal serum concentration when the dams were injected with radiolabeled chromium trichloride in mid-gestation and 0.8% of the maternal serum radioactivity concentration when injected in late gestation. Radioactivity from both treatments accumulated in fetal skeletons in calcified areas and in the yolk sac placenta (Danielsson et al. 1982). Danielsson et al. (1982) noted that the radioactivity after administration of chromium(VI) may represent chromium(III) after reduction in the tissues. Chromium(III) also crossed the placenta of mice injected intraperitoneally with chromium trichloride (Iijima et al. 1983). While the results indicate that both chromium(VI) and chromium(III) may pose developmental hazards, they cannot be used to indicate that exposure of pregnant animals to chromium(III) by inhalation or oral routes would result in significant placental transfer because chromium(III) compounds are not well absorbed by these routes (see Section 3.4.1).

Tissue distribution in rats and mice after 14 days of intraperitoneal injection of 0.8 mg chromium(VI)/day as potassium chromate were: liver 6.00 μg/g wet weight in rats and 8.89 in mice, kidney 24.14 and 11.77, spleen 15.26 and 6.92, femur 6.53 and 6.30, lung 3.99 and 2.89, heart 3.13 and 1.75, muscle 1.10 and 0.51, and blood 4.52 and 1.56. (Kargacin et al. 1993). Kidney and blood chromium concentrations were 2- and 4-fold higher, respectively, in rats compared to mice. Red blood cell concentrations were 3-fold higher in rats than mice and hemoglobin binding of chromium was twice as high in rats. By contrast, after oral exposure levels, in blood for rats and mice were similar. The authors ascribed this to faster entry into the blood after intraperitoneal injection and thus a greater likelihood that chromium(VI) could be sequestered in rat erythrocytes by reduction.

3.4.3 Metabolism

Chromium(III) compounds are essential to normal glucose, protein, and fat metabolism. In addition, chromium(III) is capable of forming complexes with nucleic acids and proteins. Chromium(III) may also participate in intracellular reduction and oxidation reactions. Chromium(VI) is unstable inside the body and is ultimately reduced to chromium(III) *in vivo* by a variety of reducing agents. Chromium(V) and chromium(IV) are transient intermediates in this process.

Currently, the biological target for the essential effects of chromium(III) is unknown. Chromodulin, also referred to as GTF, has been proposed as one possible candidate (Jacquamet et al. 2003). The function of chromodulin, an oligopeptide complex containing with four chromic ions, has not been established; however, a possible mechanism is that chromodulin facilitates the interaction of insulin with its cellular receptor sites, although this has not been proven (Anderson 1998a, 2003; IOM 2001).

In vivo and *in vitro* experiments in rats indicated that, in the lungs, chromium(VI) can be reduced to chromium(III) by ascorbate. The reduction of chromium(VI) by ascorbate results in a shorter residence time of chromium in the lungs and constitutes the first defense against oxidizing reagents in the lungs. When ascorbate is depleted from the lungs, chromium(VI) can also be reduced by glutathione. The level of ascorbic acid in the adult human lung has been estimated as approximately 7 mg/100 g wet tissue (Hornig 1975). The reduction of chromium(VI) by glutathione is slower and results in greater residence time of chromium in the lungs, compared to reduction by ascorbate (Suzuki and Fukuda 1990). Other studies reported the reduction of chromium(VI) to chromium(III) by epithelial lining fluid (ELF) obtained from the lungs of 15 individuals by bronchial lavage. The average reduction accounted for 0.6 µg chromium(VI)/mg of ELF protein. In addition, cell extracts made from pulmonary alveolar macrophages derived from five healthy male volunteers were able to reduce an average of 4.8 µg chromium(VI)/10^6 cells or 14.4 µg chromium(VI)/mg protein (Petrilli et al. 1986b). Metabolism of the chromium(VI) to chromium(III) by these cell fractions significantly reduced the mutagenic potency of the chromium when tested in the Ames reversion assay. Postmitochondrial (S12) preparations of human lung cells (peripheral lung parenchyma and bronchial preparations) were also able to reduce chromium(VI) to chromium(III) (De Flora et al. 1984). Moreover, large individual differences were observed (De Flora et al. 1984, 1987b), and extracts from pulmonary alveolar macrophages of smokers reduced significantly more chromium(VI) to chromium(III) than extracts from cells of nonsmokers. Because chromium(III) does not readily enter cells, these data suggest that reduction of chromium(VI) by the ELF may constitute the first

line of defense against toxicity of inhaled chromium compounds. Furthermore, uptake and reduction of chromium compounds by the pulmonary alveolar macrophages may constitute a second line of defense against pulmonary toxicity of chromium(VI) compounds. Microsomal reduction of chromium(VI) occurs in the lungs mainly as it does in the liver, as discussed below.

The first defense against chromium(VI) after oral exposure is the reduction of chromium(VI) to chromium(III) in the gastric environment where gastric juice (De Flora et al. 1987a) and ascorbate (Samitz 1970) play important roles. Studies using low-frequency electron paramagnetic resonance (EPR) spectrometry have shown that chromium(VI) is reduced to chromium(V) *in vivo* (Liu et al. 1994, 1995, 1997a, 1997b; Ueno et al. 1995b). *In vitro*, low concentrations of ascorbate favor the formation of chromium(V), whereas higher concentrations of ascorbate favor the formation of the reduced oxidation state, chromium(III) (Liu et al. 1995). EPR spectrometric monitoring also showed that chromium(VI) was rapidly reduced to chromium(V) on the skin of rats, with a 3-fold greater response when the stratum corneum was removed (Liu et al. 1997a). Thus, dermal effects from direct skin contact with chromium(VI) compounds may be mediated by rapid reduction to chromium(V). In whole blood and plasma, increasing ascorbate levels led to an increased oxidation of chromium(VI) to chromium(III) (Capellmann and Bolt 1992).

For humans, the overall chromium(VI)-reducing/sequestering capacities were estimated to be 0.7–2.1 mg/day for saliva, 8.3–12.5 mg/day for gastric juice, 11–24 mg for intestinal bacteria eliminated daily with feces, 3,300 mg/hour for liver, 234 mg/hour for males and 187 mg/hour for females for whole blood, 128 mg/hour for males and 93 mg/hour for females for red blood cells, 0.1–1.8 mg/hour for ELF, 136 mg/hour for pulmonary alveolar macrophages, and 260 mg/hour for peripheral lung parenchyma. Although these *ex vivo* data provide important information in the conversion of chromium(VI) to reduced states, the values may over- or underestimate the *in vivo* reducing capabilities (De Flora et al. 1997).

Reduction of chromium(VI) in the red blood cell occurs by the action of glutathione. Since the red blood cell membrane is permeable to chromium(VI) but not chromium(III), the chromium(III) formed by reduction of chromium(VI) by glutathione is essentially trapped within the erythrocyte for the life-span of the cell (Paustenbach et al. 2003), with approximately 1% of chromium eluting from the erythrocyte daily (ICSH 1980). Eventually, the diffusion of chromium(VI), the reduction to chromium(III), and complexing to nucleic acids and proteins within the cell will cause the concentration equilibrium to change so that more chromium(VI) is diffused through the membrane. Thus, there is a physiological drag so that increased diffusion results in greater chromium concentrations in the cell (Aaseth et al. 1982). It

appears that the rate of uptake of chromium(VI) by red blood cells may not exceed the rate at which they reduce chromium(VI) to chromium(III) (Corbett et al. 1998). *In vitro* incubation of red blood cells with an excess of sodium chromate(VI) (10 mM) decreased glutathione levels to 10% of the original amount (Wiegand et al. 1984). The above concepts are applicable to the uptake and reduction of chromium(VI) in other cell types.

The effect of glutathione-depleting agents on the amounts of cellular chromium(III) and chromium(V) was determined in Chinese hamster V-79 cells treated with sodium chromate (Sugiyama and Tsuzuki 1994). Buthionine sulfoximine at 25 μM reduced glutatione levels to about 1% of control values, and increased chromium(V) levels by about 67%. The total chromium uptake was decreased by about 20% and chromium(III) levels were decreased by 20%. Diethylmaleate (1 mM) decreased glutathione levels to <1%, decreased chromium(V) levels by 27% and chromium(III) levels by 31%. However, the cellular uptake of total chromium was decreased to nearly 46%. The authors explained that the reason that the diethylmaleate inhibited the reduction of chromium(VI) to both chromium(III) and chromium(V) was not due to the decreased uptake, but involved the inhibition of the chromate-reducing enzymes in the cell.

In addition to the reduction of chromium(VI) by ascorbate or glutathione, *in vitro* studies have demonstrated reduction of chromium(VI) by microsomal enzymes. Hepatic microsomal proteins from male Sprague-Dawley rats pretreated with chromium(VI) reduced chromium(VI) to chromium(III). The rate of reduction varied both with the concentration of microsomal protein and the concentration of nicotinamide adenine dinucleotide phosphate (NADPH). In the absence of NADPH, microsomes did not reduce significant amounts of chromium(VI) over the 24-hour observation period. Therefore, the reduction of chromium(VI) in rat hepatic microsomes is NADPH-dependent (Gruber and Jennette 1978). Another study followed the kinetics of chromium(VI) reduction in hepatic microsomes from rats (Garcia and Jennette 1981). Induction of cytochrome P448 enzymes had no effect on the kinetics of the reaction, while induction of cytochrome P450 and NADPH-cytochrome P450 reductase resulted in a decrease in the apparent chromate-enzyme dissociation constant, and an increase in the apparent second-order rate constant, and no change in the apparent turnover number. Inhibition of NADPH-cytochrome P450 reductase and NADH-cytochrome b_5 reductase inhibited the rate of microsomal reduction of chromium(VI), as did the addition of specific inhibitors of cytochrome P450. The results demonstrate the involvement of cytochrome P450, NADPH-dependent-cytochrome P450 reductase, and to a lesser extent cytochrome b_5 and NADH-dependent-cytochrome b_5 reductase, in the reduction of chromate by rat hepatic microsomes. The conversion of chromium(VI) to chromium(III) in rats can occur by electron transfer through cytochrome P450 and cytochrome b_5. Both oxygen and carbon monoxide were found to

inhibit the *in vitro* cytochrome P450 and cytochrome b_5-dependent reduction of chromium(VI) (Mikalsen et al. 1989). The assertion that cytochrome P450 is involved in the reduction of chromium(VI) to chromium(III) has been further strengthened by Petrilli et al. (1985), who demonstrated that inducers of cytochrome P450 can increase the conversion of chromium(VI) to chromium(III) in S-9 mixtures prepared from the liver and lungs of exposed rats. Furthermore, it was observed that chromium(VI) can induce pulmonary cytochrome P450 and thus its own reduction in the lungs (Petrilli et al. 1985). Chromium(VI) apparently can alter the P450 activity in isolated rat microsomes. Witmer et al. (1994) demonstrated that hepatic microsomes from male rats treated with chromium(VI) resulted in a significant decrease in hydroxylation of testosterone at the 6β, 16α, and 2α positions, indicating a decrease in the activity of P4503A1 and 3A2. In lung microsomes, an increased hydroxylation was observed at the 16α and 16β positions, indicating an increase in P450IIB1 activity. However, hepatic microsomes from treated females showed a 4-to5-fold increase in hydroxylation activity of testosterone at the 6β position, which demonstrated that the metabolic effects of chromium differ between males and females.

Two studies have examined possible species differences in the ability of microsomes to reduce chromium(VI) (Myers and Myers 1998; Pratt and Myers 1993). Chromium(VI) reduction was enzymatic and NADPH-dependent, and the rates were proportional to the amount of microsome added. In humans, the K_m for chromium(VI) was 1–3 orders of magnitude lower than K_m values in rats, although the V_{max} was similar. This suggests that the human liver has a much greater capacity to reduce chromium(VI) than the rat liver. Also contrary to the rodent data, oxygen and cytochrome P450 inhibitors (carbon monoxide, piperonyl butoxide, metyrapone, and aminopyrine) did not inhibit chromium(VI) reduction. These differences indicate that, in humans, cytochrome P450 does not play a significant role in the reduction process, but that other microsomal flavoproteins are responsible for reducing chromium(VI). Inhibition of flavoproteins by $TlCl_3$ decreased chromium(VI) reduction by 96–100%, while inhibition of cytochrome c reductase (P450 reductase) by bromo-4'-nitroacetophenone resulted in an 80–85% inhibition of chromium(VI) reduction. Combined, these observations implicate P450 reductase, working independently of cytochrome P450, as a major contributor in the reduction of chromium(VI) in human microsomes. These findings suggest that metabolism of chromium(VI) in rodent systems may not readily be extrapolated to humans.

Microsomal reduction of chromium(VI) can also result in the formation of chromium(V), which involves a one-electron transfer from the microsomal electron-transport cytochrome P450 system in rats. The chromium(V) complexes are characterized as labile and reactive. These chromium(V) intermediates persist for 1 hour *in vitro*, making them likely to interact with deoxyribonucleic acid (DNA), which may

eventually lead to cancer (Jennette 1982). Because chromium(V) complexes are labile and reactive, detection of chromium(V) after *in vivo* exposure to chromium(VI) was difficult in the past. More recently, Liu et al. (1994) have demonstrated that chromium(V) is formed *in vivo* by using low-frequency electron paramagnetic resonance (EPR) spectroscopy on whole mice. In mice injected with sodium dichromate(VI) intravenously into the tail vein, maximum levels of chromium(V) were detected within 10 minutes and declined slowly with a life-time of about 37 minutes. The time to reach peak *in vivo* levels of chromium(V) decreased, in a linear manner as the administered dose levels of sodium dichromate decreased. The relative tissue distributions of chromium(V) indicated that most was found in the liver and much lesser amounts in blood. None was detected in kidney, spleen, heart, or lung. When the mice were pretreated with metal ion chelators, the intensity of the EPR signal decreased demonstrating that the formation of chromium(V) was inhibited. Reactions of chromium(VI) with glutathione produced two chromium(V) complexes and a glutathione thiyl radical. Reactions of chromium(VI) with DNA in the presence of glutathione produced chromium-DNA adducts. The level of chromium-DNA adduct formation correlated with chromium(V) formation. The reaction of chromium(VI) with hydrogen peroxide produced hydroxyl radicals. Reactions of chromium(VI) with DNA in the presence of high concentrations of hydrogen peroxide (millimolar compared to 10^{-7}–10^{-9} M inside cells) produced significant DNA strand breakage and the 8-hydroxy guanosine adduct, which correlated with hydroxyl radical production (Aiyar et al. 1989, 1991). Very little chromium(V) was generated by this pathway. It was postulated that the reaction of chromium(VI) with hydrogen peroxide produces tetraperoxochromium(V) species that act as a catalyst in a Fenton-type reaction producing hydroxyl radicals in which chromium(V) is continuously being recycled back to chromium(VI). The regeneration of chromium(VI) through interactions with chromium(V) and hydrogen peroxide is consistent with the findings of Molyneux and Davies (1995) (see Section 3.5.2). As discussed above, chromium(VI) is ultimately reduced to chromium(III) within the cell. Chromium(III) can form stable complexes with DNA and protein (De Flora and Wetterhahn 1989), which is discussed further in Section 3.5.2.

The mechanism for clearance of chromium(VI) once reduced inside the liver cell may involve a chromium(III)-glutathione complex. The glutathione complex would be soluble through the cell membrane and capable of entering the bile (Norseth et al. 1982). The complexing of chromium(III) to other ligands has been shown to make them more permeable to the cell membrane (Warren et al. 1981).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Normal urinary levels of chromium in humans have been reported to range from 0.22 to 1.8 μg/L (0.00024–0.0018 mg/L) with a median level of 0.4 μg/L (0.0004 mg/L) (IOM 2001; Iyengar and Woittiez 1988). Humans exposed to 0.05–1.7 mg chromium(III)/m^3 as chromium sulfate and 0.01–0.1 mg chromium(VI)/m^3 as potassium dichromate (8-hour TWA) had urinary excretion levels of 0.0247–0.037 mg chromium(III)/L. Workers exposed mainly to chromium(VI) compounds had higher urinary chromium levels than workers exposed primarily to chromium(III) compounds. An analysis of the urine did not detect the hexavalent form of chromium, indicating that chromium(VI) was rapidly reduced before excretion (Cavalleri and Minoia 1985; Minoia and Cavalleri 1988). Chromium(III) compounds were excreted rapidly in the urine of workers, following inhalation exposure to chromium(III) as chromium lignosulfonate. Workers exposed to 0.005–0.23 mg chromium(III)/m^3 had urine concentrations of 0.011–0.017 mg chromium(III)/L. The half-time for urinary excretion of chromium was short, 4–10 hours, based on an open, one-compartment kinetic model (Kiilunen et al. 1983). Tannery workers had higher urinary chromium(III) concentrations in postshift urine samples taken Friday afternoon and in preshift urine samples taken Monday, compared to controls. These workers also had hair concentrations of chromium that correlated with urinary levels. Analysis of workroom air revealed no detectable chromium(VI) and 0.0017 mg chromium(III)/m^3 (time-weighted average) (Randall and Gibson 1987). Elimination of chromium(III) from hair, serum, and urine has been studied in a group of 5 men who had ceased working in a leather tannery 9 months earlier (Simpson and Gibson 1992). Compared to levels recorded during employment, the mean level of chromium in hair was reduced from 28.5 to 2.9 μmol/g; serum levels were reduced from 9.4 to 3.8 nmol/L. These levels are comparable to those in the general population. Urine levels were unchanged (13.8 nmol/L while working and 14.4 nmol/L 9 months later); the authors stated that this was probably caused by consumption of beer (a source of chromium) the night before sampling. Data from autopsy studies indicate that chromium can be retained in the lung for decades following cessation of occupational exposures (Brune et al. 1980; Hyodo et al. 1980; Mancuso 1997b).

Peak urinary chromium concentrations were observed at 6 hours (the first time point examined) in rats exposed intratracheally to 0.44 mg/kg chromium(III) as chromium acetate hydroxide or chromium(VI) as sodium dichromate (Gao et al. 1993). Chromium urinary concentrations decreased rapidly, falling from 4,535 μg chromium/g creatinine at 6 hours to 148 μg chromium/g at 72 hours for the chromium acetate

hydroxide and from 2,947 μg chromium/g creatinine at 6 hours to 339 μg chromium/g at 72 hours for sodium dichromate.

Elimination of chromium was very slow in rats exposed to 2.1 mg chromium(VI)/m^3 as zinc chromate 6 hours/day for 4 days. Urinary levels of chromium remained almost constant for 4 days after exposure and then decreased, indicating that chromium bound inside the erythrocyte is released slowly (Langård et al. 1978).

3.4.4.2 Oral Exposure

Given the low absorption of chromium compounds by the oral route, the major pathway of excretion after oral exposure is through the feces.

An acute, oral dose of radioactive chromium(III) as chromium chloride or chromium(VI) as sodium chromate was administered to humans after which feces and urine were collected for 24 hours and 6 days, respectively, and analyzed for chromium. The amount of chromium in the 6-day fecal collection was 99.6 and 89.4% of the dose for chromium(III) and chromium(VI) compounds, respectively. The amount of chromium in the 24-hour urine collection was 0.5 and 2.1% of the dose for chromium(III) and chromium(VI) compounds, respectively (Donaldson and Barreras 1966). In subjects drinking 0.001–0.1 mg chromium(VI)/kg/day as potassium chromate in water for 3 days, <2–8% of the dose was excreted in the urine (Finley et al. 1997). The percentage of the dose excreted appeared to increase with increasing dose.

Urinary excretion rates have been measured in humans after oral exposure to several chromium compounds (Finley et al. 1996b). A group of four male and two female volunteers ingested capsules containing chromium(III) picolinate at a dose of 200 μg/day for 7 days, to ensure that chromium deficiency was not a confounding factor. They then ingested 0.005 mg/kg/day chromium(VI) as potassium chromate (3 days), and 1.0 mg/kg/day chromium(III) as chromic oxide (3 days), with 3 days without dosing between the potassium chromate and chromic oxide doses. Urinary excretion rates of chromium were significantly elevated compared to postdosing control levels after seven daily doses of chromium(III) picolinate (2.4±0.8 versus 0.75±0.53 μg/day). The excretion rate increased sharply on the first of 3 days of potassium chromate dosing (11±17 μg/day) and remained steady over the next 2 days (13–14 μg/day). Excretion rates fell to 2.5±0.72 during 2 days without dosing and continued to fall during the 3 days of chromic oxide dosing, reaching rates similar to those seen postdosing. Mean pooled

urinary concentrations during the dosing periods were 2.4 μg chromium/g creatinine from exposure to chromium(VI) and 0.4 μg chromium/g creatinine from exposure to chromium(III) as compared to 0.23 μg chromium/g creatinine during the postdosing time periods. The lower urinary excretion of chromium(III) after exposure to chromic oxide reflects the poorer absorption of inorganic chromium(III) compounds compared to inorganic chromium(VI) compounds.

The half-life for chromium urinary excretion after administration in drinking water as potassium dichromate has been estimated in humans (Kerger et al. 1997). Ingestion of 0.05 mg chromium(VI)/kg resulted in an extended time course of excretion. Approximately 76–82% of the 14-day total amount of chromium in the urine was excreted within the first 4 days (mean peak concentration 209 μg chromium/g creatinine; range 29–585 μg chromium/g creatinine). The average urinary excretion half-life for four of the volunteers was 39 hours at this dose. All subjects had returned to background concentrations (0.5–2.0 μg chromium/g creatinine) by 14 days postdosing. About 87% of the total amount of chromium in the urine measured over 8 days was excreted during the first 4 days for one volunteer ingesting 0.03 mg chromium(VI)/kg (peak 97 μg chromium/g creatinine on day of ingestion). Urinary chromium concentrations had returned to an average of 2.5 μg chromium/g creatinine within 7 days postdosing, the last time point measured. Urinary excretion half-life in this volunteer was 37 hours. Similar time courses of excretion were observed when volunteers took the same doses as daily doses over 3-day periods. An earlier study by this group (Kerger et al. 1996a) examined urinary excretion half-lives following a bolus dose of 10 ppm (approximately 0.06 mg chromium/kg) chromium(III) chloride, potassium dichromate reduced with orange juice (presumably, the juice reduced the potassium dichromate to chromium(III)-organic complexes and chromium(III) ions), or potassium dichromate. The calculated urinary excretion half-lives for the three chromium solutions were 10.3, 15, and 39.3 hours, respectively. The potassium dichromate half-life is consistent with the results from the Kerger et al. (1997) study. If, in these studies, all of the absorbed chromium(VI) was rapidly and completely converted to chromium(III), there should be no difference in urinary half-life. The difference in excretion half-lives following dosing with chromium(III) and chromium(VI) appears to reflect incomplete reduction of absorbed chromium(VI) to chromium(III) as well as longer retention of chromium(VI) in tissues. The prolonged half-life following dosing with chromium(VI) appears to be a composite of the half-lives the chromium(VI) and chromium(III) derived from the reduction of chromium(VI) in the blood. Given that most is converted to chromium(III), the half-life for the sequestered chromium is quite long (much longer than 40 hours) and reflects the half-life of chromium observed in the red blood cells. Pretreatment of chromium(VI) with orange juice apparently did not convert all chromium(VI) to chromium(III), as indicated by a half-life of 15 hours.

The urinary excretion kinetics of chromium have also been examined in eight adults that were administered chromium(III) at 400 μg/day as chromium(III) picolinate for 3 consecutive days (Gargas et al. 1994). The mean time to peak urinary concentration was 7.18±2.11 hours (range 2.9–13.0 hours), the mean peak concentration being 7.92±4.24 μg chromium/g creatinine (range 3.58–19.13 μg/g creatinine). Excretion diminished rapidly after the peak, but did not appear to return to background in most of the volunteers before the next daily dose.

Pharmacokinetic models were used to predict the retention and excretion of ingested chromium(III) picolinate (Stearns et al. 1995a). A single dose of 5.01 mg (assuming 2.8% or 140 μg of the chromium(III) picolinate is absorbed) resulted in 11 μg (7.9%) retained after 1 year. The model predicted that about 1.4 μg would still be present in body tissues 10 years after dosing, and continuous dosing over a 1-year period would result in 6.2 mg of chromium(III) picolinate being retained, requiring about 20 years to reduce the retained level to 0.046 mg. These projected retention estimates may be 2–4-fold lower than results obtained from actual clinical findings. The authors caution that accumulative daily intake of chromium(III) may result in tissue concentrations that could be genotoxic.

Daily urinary excretion levels of chromium were nearly identical in men and women (averages of 0.17 and 0.20 μg/L, respectively; 0.18 μg/L combined) who ate normal dietary levels of chromium (≈60 μg chromium(III)/day). When the subjects' normal diets were supplemented with 200 μg chromium(III)/day as chromium trichloride to provide intakes of ≈260 μg chromium(III)/day, urinary excretion of chromium rose proportionately to an average of 0.98 μg/L combined. Thus a 5-fold increase in oral intake resulted in about a five-fold increase in excretion, indicating absorption was proportional to the dose regardless of whether the source was food or supplement (Anderson et al. 1983). A group of 23 elderly subjects who received an average of 24.5 μg/day (0.00035 mg chromium(III)/kg/day) from their normal diets excreted 0.4 μg chromium/day in the urine (1.6%) and 23.9 μg chromium/day in the feces (97.6%), with a net retention of 0.2 μg/day (0.8%). Based on the 1980 daily requirement for absorbable chromium of 1 μg/day by the National Academy of Science Food and Nutrition Board, the retention was considered adequate for their requirements (Bunker et al. 1984).

An estimate of the half-life of elimination from plasma has been reported in humans. Uptake of potassium dichromate was determined in a man who was given 0.8 mg of chromium(VI) in drinking water 5 times each day for 17 days (Paustenbach et al. 1996). Steady-state concentrations of chromium in blood were attained after 7 days and a plasma elimination half-life of 36 hours was estimated.

Measurement of the chromium content in 255 milk samples from 45 lactating American women revealed that most samples contained <0.4 μg/L with a mean value of 0.3 μg/L (Casey and Hambidge 1984). Another study (Anderson et al. 1993) measured chromium levels in the breast milk of 17 women 60 days postpartum, and reported mean levels of ~0.2 μg/L. Lactation, therefore, represents a route of excretion of chromium and a potential route of exposure to the nursing infant. However, the precise relationship between maternal chromium levels and levels in breast milk is unclear, if such a relationship exists at all (Anderson et al. 1993; Engelhardt et al. 1990; Mohamedshah et al. 1998).

Chromium can be excreted in hair and fingernails. Mean trace levels of chromium detected in the hair of individuals from the general population of several countries were as follows: United States, 0.23 ppm; Canada, 0.35 ppm; Poland, 0.27 ppm; Japan, 0.23 ppm; and India, 1.02 ppm (Takagi et al. 1986). Mean levels of chromium in the fingernails of these populations were: United States, 0.52 ppm; Canada, 0.82 ppm; Poland, 0.52 ppm; Japan, 1.4 ppm; and India, 1.3 ppm (Takagi et al. 1988).

Rats given 18 mg chromium(VI)/kg as potassium dichromate by gavage excreted about 25 μg chromium in the first 24 hours after dosing and ≈10 μg chromium in each of the next 24-hour periods (Banner et al. 1986).

In rats and hamsters fed chromium compounds, fecal excretion of chromium varied slightly from 97 to 99% of the administered dose. Urinary excretion of chromium varied from 0.6 to 1.4% of the dose administered as either chromium(III) or chromium(VI) compounds (Donaldson and Barreras 1966; Henderson et al. 1979; Sayato et al. 1980). The urinary and fecal excretion over 2-day periods in rats treated for 8 days by gavage with 13.92 mg chromium/kg/day in corn oil was higher when soil containing 70% chromium(III) and 30% chromium(VI) was the source of chromium than when chromium(VI) as calcium chromate was the source (see Section 3.4.2.2). Total urinary and fecal excretion of chromium on days 1 and 2 of dosing were 1.8 and 19%, respectively, of the dose from soil and <0.5 and 1.8%, respectively, of the dose from calcium chromate. Total urinary and fecal excretion of chromium on days 7 and 8 of dosing were higher than on days 1 and 2. For contaminated soil, urinary excretion was 1.12% and fecal excretion was 40.6% of the dose. For calcium chromate, urinary excretion was 0.21% and fecal excretion was 12.35% of the dose (Witmer et al. 1991). Whether the higher excretion of chromium after dosing with soil than with the chromate salt represents greater bioavailability from soil could not be determined because about 50% of the administered dose could not be accounted for from the excretion and distribution data (see Section 3.4.2.2). Excretion of chromium(III) in dogs was approximately equal

to the clearance of creatinine, indicating little tubular absorption or reabsorption of chromium in the kidneys (Donaldson et al. 1984).

3.4.4.3 Dermal Exposure

Information regarding the excretion of chromium in humans after dermal exposure to chromium or its compounds is limited. Fourteen days after application of a salve containing potassium chromate(VI), which resulted in skin necrosis and sloughing at the application site, chromium was found at 8 mg/L in the urine and 0.61 mg/100 g in the feces of one individual (Brieger 1920). A slight increase (over background levels) in urinary chromium levels was observed in four subjects submersed in a tub of chlorinated water containing 22 mg chromium(VI)/L as potassium dichromate(VI) for 3 hours (Corbett et al. 1997). For three of the four subjects, the increase in urinary chromium excretion was <1 μg/day over the 5-day collection period.

^{51}Chromium was detected in the urine of guinea pigs after radiolabeled sodium chromate(VI) or chromium(III) trichloride solutions were placed over skin depots that were monitored by scintillation counting to determine the dermal absorption (Wahlberg and Skog 1965).

3.4.4.4 Other Routes of Exposure

Elevated levels of chromium in blood, serum, urine, and other tissues and organs have been observed in patients with cobalt-chromium knee and hip arthroplasts (Michel et al. 1987; Sunderman et al. 1989). Whether corrosion or wear of the implant can release chromium (or other metal components) into the systemic circulation depends on the nature of the device. In one study, the mean postoperative blood and urine levels of chromium of nine patients with total hip replacements made from a cast cobalt-chromium-molybdenum alloy were 3.9 and 6.2 μg/L, respectively, compared with preoperative blood and urine levels of 1.4 and 0.4 μg/L, respectively. High blood and urinary levels of chromium persisted when measured at intervals over a year or more after surgery. These data suggest significant wear or corrosion of the metal components. No significant difference was found for patients with hip replacements made from the alloy and articulated with polyethylene (Coleman et al. 1973). Similarly, serum and urinary levels of chromium in patients with implants made from a porous coated cobalt chromium alloy with polyethylene components (to prevent metal-to-metal contact) were not significantly different from patients with implants made without chromium (Sunderman et al. 1989).

A number of factors have been shown to alter the rate of excretion of chromium in humans. Intravenous injection of calcium EDTA resulted in a rapid increase in the urinary excretion of chromium in metal workers (Sata et al. 1998). Both acute and chronic exercises have been shown to increase chromium excretion in the urine, though the increased excretion did not appear to be accompanied with decreased levels of total native chromium (Rubin et al. 1998). An increased rate of chromium excretion has been reported in women in the first 26 weeks of pregnancy (Morris et al. 1995b). Chromium supplementation did not appear to alter the rate of excretion into breast milk in postpartum women (Mohamedshah et al. 1998).

The urinary excretion of chromium after a single or during repeated subcutaneous injections of potassium dichromate was followed in rats. Following a single dose of 5.35 mg chromium(VI)/kg, chromium was excreted rapidly in two phases and was essentially complete at 48 hours. The filtered chromium load rose considerably during the first few hours after dosing and exceeded the tubular reabsorption rate. This increase was followed by a decrease that paralleled the urinary excretion of chromium. During repeated injections with 1.05 mg chromium(VI)/kg/day, every other day for 12 weeks, urinary excretion and diffusible chromium renal clearance rose at relatively high parallel rates, and reached plateaus at 10 ng/min for urinary excretion and 550 µL/minute for renal clearance. The filtered load increased slightly. Since high levels of chromium were found in the renal cortex (see Section 3.4.2.4), the tubular reabsorption appeared to be limited by the accumulation of chromium in the tubular epithelium (Mutti et al. 1979).

Rats given a subcutaneous injection of potassium dichromate (chromium(VI)) and chromium nitrate (chromium(III)) excreted 36% of the chromium(VI) dose in urine and 13.9% in the feces within 7 days; 8 and 24.2% of the chromium(III) was excreted in the urine and feces within the same time period, respectively (Yamaguchi et al. 1983). Within 4 days after an intravenous dose of ^{51}chromium as chromium(III) chloride at 3 mg/kg chromium, rats excreted 5.23% of the dose in the feces and 16.3% in the urine (Gregus and Klaassen 1986).

In rats treated by intravenous injection with ^{51}chromium-labeled sodium chromate (chromium(VI)) or chromium(III) trichloride at 0.0003 or 0.345 mg chromium/kg, the bile contained 2–2.5% of the dose following chromium(VI) exposure; however, after chromium(III) exposure the concentration in the bile was ≈50 times lower (Manzo et al. 1983). Similarly, 3.5–8.4% of chromium(VI) compounds was excreted in the bile as chromium(III), compared to 0.1–0.5% of chromium(III) compounds, after intravenous injection in rats (Cikrt and Bencko 1979; Norseth et al. 1982). Administration of

diethylmaleate, which depletes glutathione, resulted in only chromium(VI) in the bile after injection of sodium chromate.

Two hours after dosing rats intravenously with potassium dichromate at 0.45–4.5 mg chromium(VI)/kg, 1.4–2.2% of the chromium was recovered in the bile. Less than 1% of the total measurable chromium in the bile was identified as chromium(VI) compounds (Cavalleri et al. 1985).

Male Swiss mice exposed to 52 mg chromium(III)/kg as chromium chloride by single intraperitoneal injection or subcutaneous injection had plasma clearance half-times of 41.2 and 30.6 hours, respectively. In each case, blood levels reached control levels by 6–10 days (Sipowicz et al. 1997).

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

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

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

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of

toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

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

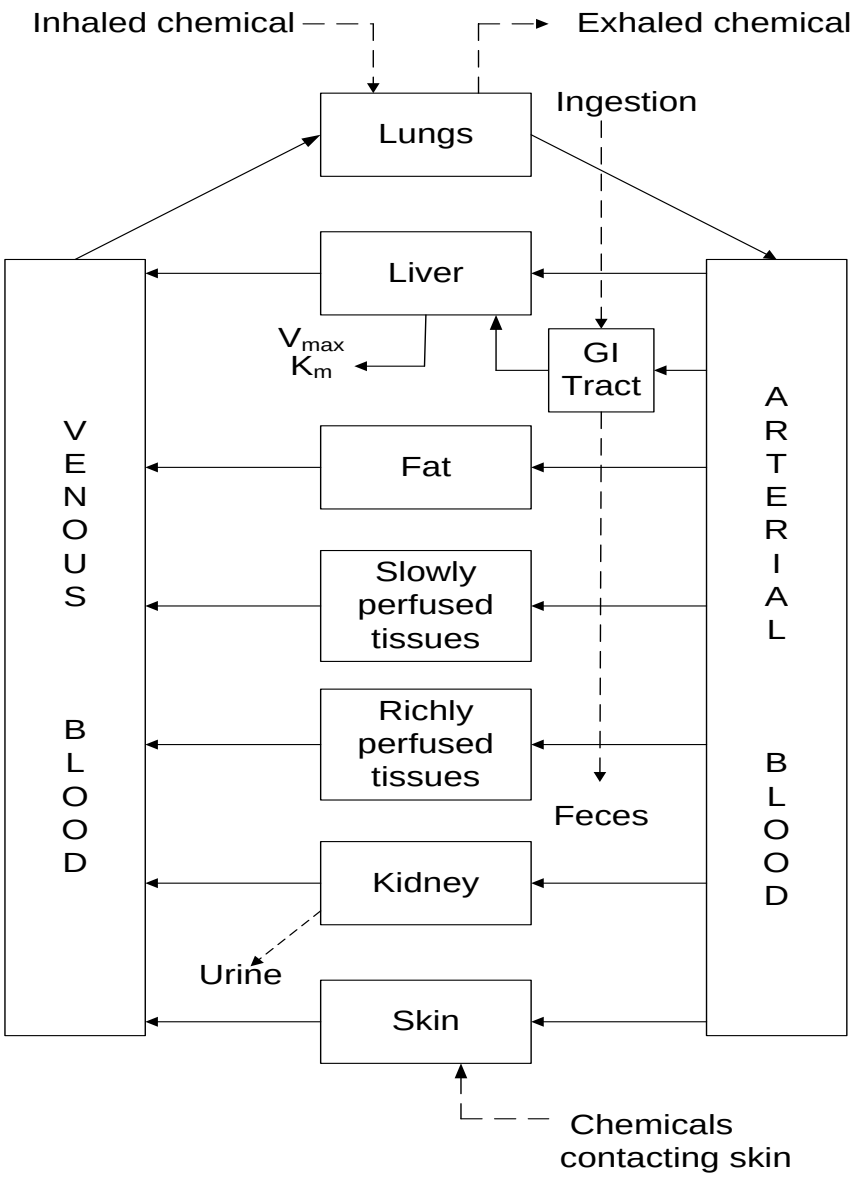
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-5 shows a conceptualized representation of a PBPK model.

PBPK models for chromium are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations. Two PBPK models for chromium have been reported that simulated developed by O'Flaherty absorption, distribution, metabolism, elimination, and excretion of chromium(III) and chromium(VI) compounds in the rat (O'Flaherty 1993c, 1996) and human (O'Flaherty et al. 2001).

3.4.5.1 O'Flaherty Model (1993a, 1996, 2001)

The structure of the O'Flaherty model is depicted in Figure 3-6. Values for chromium parameters in the rat and human model are presented in Table 3-10. The model includes compartments representing bone, kidney, liver, gastrointestinal tract, plasma, poorly-perfused tissues (e.g., muscle, skin), red blood cells, respiratory tract, and well-perfused tissues (e.g., brain, heart, lung, viscera). Chromium(VI) is assumed to

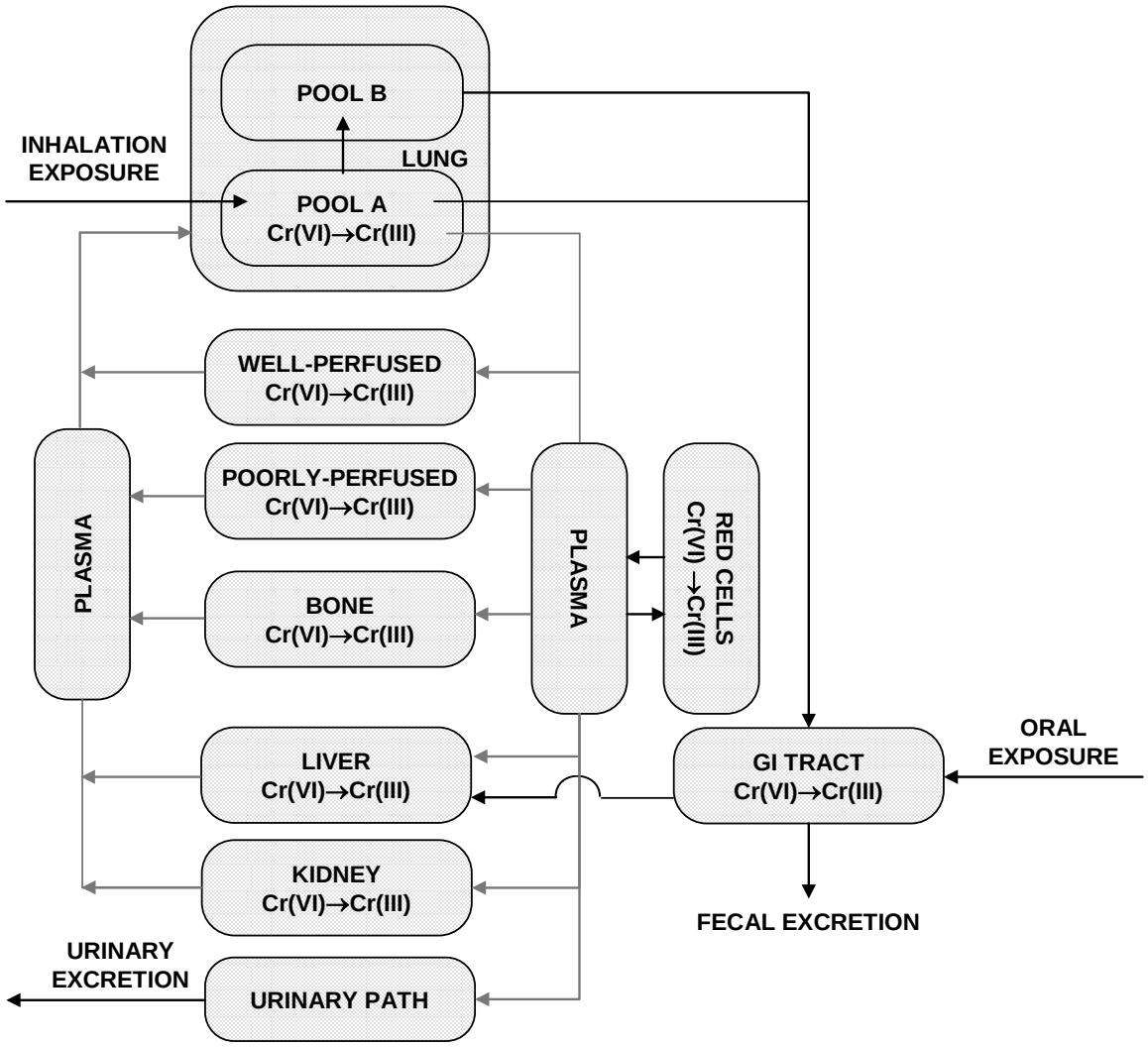
Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



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

Source: adapted from Krishnan and Andersen 1994

Figure 3-6. A Physiologically Based Model of Chromium Kinetics in the Rat



Source: O'Flaherty et al. 1996

Table 3-10. Chemical-specific Parameters in the Rat and Human Chromium Models

	Rat		Human		
Parameter[a]	Cr(IIII)	Cr(VI)	Cr(IIII)	Cr(VI)	Definition
Absorption					
KGI	0.01	0.04	0.25	2.5	First-order rate constant for absorption from the gastrointestinal 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 gastrointestinal 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 gastrointestinal 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 gastrointestinal tract.
Distribution					
CR	5.0	15.0	NA[b]	NA[b]	Relative clearance of chromium into mineralizing bone (liters of blood plasma cleared per liter of 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)

Table 3-10. Chemical-specific Parameters in the Rat and Human Chromium Models

	Rat		Human		
Parameter[a]	Cr(IIII)	Cr(VI)	Cr(IIII)	Cr(VI)	Definition
CLEAR[b]	NA	NA	12.0	12.0	Parameter in expression for clearance from blood plasma to urine (L/day)
MAX[b]	NA	NA	0.008	0.008	Parameter in expression for clearance from blood plasma to urine (mg/day)
KM[b]	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 gastrointestinal tract
Reduction					
KREDRC	NA	0.7	NA	7.0	First-order rate constant for reduction of Cr(VI) to Cr(III) in the red cell (Da^{-1})
KREDBP	NA	NA	NA	0.2	First-order rate constant for reduction of Cr(VI) to Cr(III) in blood plasma (Da^{-1})
KREDKL	NA	NA	NA	500.0	First-order rate constant for reduction of Cr(VI) to Cr(III) in kidney (Da^{-1})
KREDGI	NA	10.0	NA	100.0	First-order rate constant for reduction of Cr(VI) to Cr(III) in gastrointestinal tract contents (Da^{-1})
KRED	NA	0.5	NA	5.0	First-order rate constant for reduction of Cr(VI) 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/day) 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)

be reduced to chromium(III) in all tissues, and in the gastrointestinal and respiratory tract. Reduction is represented as a first-order rate, with distinct rates for the red cell and gastrointestinal tract, and a single value representing all other tissues.

Absorption of chromium from the gastrointestinal tract is simulated as the sum of competing first-order processes; transfer to the liver (absorption) and transfer of unabsorbed chromium to feces. Parameter values for these two processes result in absorption of approximately 1–2% of an oral dose.

The respiratory tract is represented with two subcompartments to distinguish a bioavailable chromium (pool A) from a nonbioavailable chromium (pool B). Inhaled chromium first deposits in pool A from where it can be transferred to blood (i.e., absorption), transferred to the gastrointestinal tract (i.e., mucocilliary clearance), or transferred to pool B. Chromium in pool B is cleared to the gastrointestinal tract. Transfers within and out of the respiratory tract are represented with first-order rate constants.

Transfers of chromium between plasma and soft tissues are represented with clearance terms (i.e., L/day), where clearance is given by the first-order rate constant (k_e) for transfer and tissue volume (V, clearance=k_e x V). Distinct plasma-to-tissue and tissue-to-plasma clearance values are assigned to chromium(III) and chromium(VI), with faster clearances assumed for chromium(VI), by a factor of 3,000–10,000, compared to chromium(III). In the rat model, transfers of chromium between plasma and bone are represented with clearance constants; however, this is expanded in the human model to represent chromium uptake into bone as a function of bone formation rate, and return of chromium to plasma from bone as a function of bone resorption rate (see also O'Flaherty 1993c, 1995 for further information on the bone growth and reabsorption model).

Absorbed chromium is excreted in urine. Although a biliary secretion pathway was included in the model, flux through the pathway was subsequently set to zero, based on optimizations of the model against observations. This parameterization is equivalent to assuming that either chromium is not secreted in bile, or if it is secreted into bile, it is essentially completely (and rapidly) absorbed. Urinary excretion of chromium is represented as clearance from plasma. In the rat model, plasma-to-urine clearance was assigned a constant value. In the human model, urinary clearance is represented as a variable fraction of the glomerular filtration rate, with the fraction increasing with increasing plasma concentration (e.g., 0.7% of GFR at a concentration of 0.0001 mg/L; 40% of GFR at 0.01 mg/L).

Validation of the model. Optimization of parameter values and evaluation of the rat model are described in O'Flaherty (1996). Initial values for the rat model were established based on data reported in various intravenous, oral, or intratracheal rat studies (Bragt and van Dura 1983; Cavalleri et al. 1985; Cikrt and Bencko 1979; Edel and Sabbioni 1985; MacKenzie et al. 1959; Mertz et al. 1969; Thompson and Hollis 1958; Weber 1983). Parameter values were optimized against data on kinetics of tissue levels and chromium excretion measured in rats that received intratracheal doses of ^{51}Cr(VI) or ^{51}Cr(III) (Bragt and van Dura 1983; Edel and Sabbioni 1985; Weber 1983). The optimized rat model was evaluated by comparing predictions of blood ^{51}Cr kinetics to observations made in rats exposed 6 hours/day for 4 days to dusts of zinc [^{51}Cr]chromate (76% respirable, Langård et al. 1978). Predicted blood concentrations during exposure and postexposure kinetics agreed with observations. The model was also evaluated against data from a drinking water study in which rats were exposed to drinking water concentrations of $K_2Cr(VI)O_4$ ranging from 0.45 to 25 mg/L, or to $Cr(III)Cl_3$ at a concentration of 25 mg/L for a period of 1 year (MacKenzie et al. 1958). This was not a completely independent evaluation of the model since data from this study were used to set parameters for fractional uptake of chromium into bone. Ranges for predicted:observed ratios for terminal tissue levels in rats exposed to 0.45–25 mg chromium(VI)/L were 1.2–5 for liver, 0.3–1.2 for kidney, and 0.2–1.5 for bone (femur). The ratio for rats exposed to 25 mg chromium(III)/L were 15 for liver, 0.9 for kidney, and 2 for bone.

Optimization and evaluation of the human model is described in O'Flaherty et al. (2001). The model was optimized with data on plasma and red blood cell chromium concentrations, and urinary chromium excretion in adult subjects who ingested a single dose of chromium(III) as $CrCl_3$ or chromium(VI) as $K_2Cr_2O_7$ (Finley et al. 1997; Kerger et al. 1996a, see Section 3.4.1.2 for description of these studies). The model was evaluated against data on plasma chromium concentration kinetics and urinary excretion of chromium in a single adult subject who ingested 4 mg chromium(VI)/day as $K_2Cr_2O_7$ for 17 days (Paustenbach et al. 1996; see Section 3.4.1.2), with the only adjusted parameter being the absorption rate constant. Although the model was optimized based on data from single dose studies, it reproduced the observed steady-state plasma chromium concentration, time to steady state, and elimination kinetics following cessation of the 17-day exposure.

Risk assessment. The model accounts for most of the major features of chromium(VI) and chromium(III) absorption and kinetics, and reduction chromium(VI) to chromium(III), uptake into and retention in red blood cells, and uptake and retention in bone. The human model associated bone chromium kinetics with bone formation and resorption and provides a structure for simulating age-dependent kinetics attributable to changes in bone turnover (e.g., growth, pregnancy, senescence).

Bioavailability of chromium from environmental sources is mostly unknown, except for a few chemically defined salts.

Target tissues. The rat and human models include parameters for predicting levels of chromium(III) and chromium(VI) in plasma, red blood cells, kidney, liver, bone, gastrointestinal tract, and respiratory tract. However, the rat model was calibrated against data on the above tissues, only for single dose intratracheal or intravenous exposures. Evaluations of predictions for repeated-dose exposures have been limited to blood concentration kinetics in an acute repeated dose inhalation exposure; and for terminal bone, kidney, and liver chromium levels in a 1-year drinking water study. The human model has been calibrated against data on plasma and red blood cell chromium concentrations and urinary chromium excretion following single oral doses administered to humans. Evaluation of predictions of repeated-dose outcomes have been limited to plasma and urine chromium kinetics, based on a study of a single subject exposure to chromium(VI) in drinking water for 17 days.

Species extrapolation. Evaluation of the robustness of extrapolation of the rat or human models to other species has not been reported.

Interroute extrapolation. The rat and human models include parameters for simulating inhalation and ingestion of chromium. The rat model was calibrated against data from single-dose intratracheal or intravenous exposures, and was evaluated against repeated-dose studies of inhaled and ingested chromium. The human model was calibrated and evaluated with data from ingestion studies; evaluation of the robustness of the model for predicting chromium kinetics following exposures to other routes has not been reported.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

The absorption of inhaled chromium compounds depends on a number of factors, including physical and chemical properties of the particles (oxidation state, size, solubility) and the activity of alveolar macrophages. Chromium has been identified in the tissues of occupationally-exposed humans, suggesting that chromium can be absorbed from the lungs (Cavalleri and Minoia 1985; Gylseth et al. 1977; Kiilunen et al. 1983; Mancuso 1997b; Minoia and Cavalleri 1988; Randall and Gibson 1987; Tossavainen et al. 1980). Animal studies have also demonstrated increased amounts of chromium in the blood following inhalation or intratracheal instillation exposures (Baetjer et al. 1959b; Bragt and van

Dura 1983; Langård et al. 1978; Visek et al. 1953; Wiegand et al. 1984, 1987). Chromium(VI) is more rapidly absorbed into the bloodstream than is chromium(III) (Gao et al. 1993; Suzuki et al. 1984). Chromium that is not absorbed in the lungs may be cleared via mucociliary clearance and enter the gastrointestinal tract.

Chromium is poorly absorbed from the gastrointestinal tract; the primary site of chromium absorption appears to be the jejunum (Donaldson and Barreras 1966). The bioavailability of chromium compounds seems to be most dependent on the oxidation state of the chromium atom. However, other factors, including formulation of the chromium, can influence the extent of absorption. Inorganic chromium(III) is very poorly absorbed, with only 0.5–2.8% of dietary chromium absorbed via the gastrointestinal tract of humans (Anderson 1986; Anderson et al. 1983; Donaldson and Barreras 1966; Gargas et al. 1994; Kerger et al. 1996a; Kuykendall et al. 1996). Human studies demonstrate that chromium(VI) is effectively reduced to chromium(III) by gastric juices (De Flora et al. 1987a) and in general, chromium(VI) is better absorbed than chromium(III) following oral exposure in humans (Donaldson and Barreras 1966; Finley et al. 1996b; Kerger et al. 1996a; Kuykendall et al. 1996). Absorption efficiencies ranging from 1.7 to 6.9% have been estimated in humans (Finley et al. 1996a; Kerger et al. 1996a, 1997; Kuykendall et al. 1996). Ingestion of chromium with a meal appears to increase the absorption efficiency (Chen et al. 1973).

Both chromium(III) and chromium(VI) can penetrate human skin to some extent, especially if the skin is damaged. Following dermal exposure, chromium has been detected in the blood, feces, and urine of exposed humans (Brieger 1920), though in this study, the skin was damaged, which likely facilitated absorption. An average rate of systemic uptake of chromium(VI) in humans submersed in chlorinated water containing potassium dichromate(VI) for 3 hours was 1.5×10^{-4} μg/cm^2-hour (Corbett et al. 1997). Chromium(VI) appears to penetrate the skin faster than chromium(III) (Mali et al. 1963; Spruit and van Neer 1966; Wahlberg 1970), though many other factors may be involved, including solvent (Liden and Lundberg 1979) and concentration (Baranowska-Dutkiewicz 1981).

Absorbed chromium is carried throughout the body in the blood, eventually being distributed to all tissues. Greatest concentrations of chromium are found in the blood, liver, lung, spleen, kidney, and heart (Kaufman et al. 1970; Schroeder et al. 1962; Teraoka 1981). Because insoluble chromium is not completely cleared or absorbed following inhalation exposure, greater levels of chromium are often found in lung tissues following inhalation of chromium compounds than following other methods of exposure. Tissue levels appeared to be higher after exposure to chromium(VI) than to chromium(III). This may be due to the greater ability of chromium(VI) to cross cell membranes and may also be a function of

administration of doses high enough to overwhelm the chromium(VI) reduction mechanisms. De Flora et al. (1997) have demonstrated that liver, erythrocytes, whole blood, lung epithelial fluid, alveolar macrophages, and peripheral parenchyma cells all have the ability to reduce chromium(VI) to chromium(III). Chromium has been detected in breast milk (Casey and Hambidge 1984; Shmitova 1980), but the relationship between chromium exposure, dietary or otherwise, and breast milk chromium levels is inconclusive (Anderson et al. 1993; Engelhardt et al. 1990; Mohamedshah et al. 1998).

Systemic chromium(III) does not appear to be stored for extended periods of time within the tissues of the body. However, the prolonged half-life of chromium(VI) compared to chromium(III) in humans (Kerger et al. 1997) and animals indicate that a portion of the absorbed chromium(VI) dose that is not converted to chromium(III) is being sequestered inside cells. Single- and multiple-exposure studies in humans have shown a one-compartment clearance half-time in humans on the order of 36 hours (Kerger et al. 1997; Paustenbach et al. 1996) following oral exposure. This half-time is sufficiently long to allow for accumulation of chromium following regular repeated exposure. Following inhalation exposure, insoluble chromium that is not cleared from the lungs may remain for a considerable time. In the blood, chromium(VI) is taken up by erythrocytes and reduced to chromium(III) which forms complexes with hemoglobin and other intracellular macromolecules; these complexes are retained within the erythrocyte for the life-span of the cell (Paustenbach et al. 2003).

Inhaled chromium can be eliminated from the lungs by absorption into the bloodstream, by mucociliary clearance, and by lymphatic system clearance (Bragt and van Dura 1983; Perrault et al. 1995; Visek et al. 1953; Wiegand et al. 1984, 1987). The primary routes of elimination of absorbed chromium is urine and feces. It can also be eliminated in hair and fingernails (Randall and Gibson 1989; Stearns et al. 1995a; Takagi et al. 1986). Chromium, once reduced to chromium(III) in the liver, is then conjugated with glutathione and enters bile where it is excreted in the feces (Norseth et al. 1982). Because chromium is poorly absorbed following oral exposure, a large percentage of the amount ingested is excreted in the feces. The half-time of urinary excretion of chromium is short, 4–10 hours for inhalation exposure (Kiilunen et al. 1983), 10 hours for oral exposure to chromium(III) (Kerger et al. 1996a), and 40 hours for oral exposure to chromium(VI) (Kerger et al. 1996a, 1997). Following dermal exposure, chromium that is not absorbed into the bloodstream will remain on the skin until it is eliminated, usually by washing or other physical processes. Absorbed chromium is primarily eliminated in the urine.

3.5.2 Mechanisms of Toxicity

The toxic potency of chromium is dependent on the oxidation state of the chromium atom, with chromium(VI) more potent than chromium(III). The mechanisms of chromium toxicity and carcinogenicity are very complex. They are mediated partly through reactive intermediates during intracellular reduction of chromium(VI) to chromium(III) and oxidative reactions, and partly mediated by chromium(III) which is the final product of intracellar chromium(VI) reduction and forms deleterious complexes with critical target macromolecules (Chen and Shi 2002; Costa 2003; Costa and Klein 2006a; Ding and Shi 2002; Jeejeebhoy 1999; Levina and Lay 2005; Liu and Shi 2001; O'Brien et al. 2003; Paustenbach et al. 2003; Shrivastava et al. 2002; Zhitkovich 2005). Chromium(III) may form complexes with peptides, proteins, and DNA, resulting in DNA-protein crosslinks, DNA strand breaks, and alterations in cellular signaling pathways, which may contribute to toxicity and carcinogenicity of chromium compounds.

The greater toxic potency of chromium(VI) relative to chromium(III) most likely is related to two factors: (1) the higher redox potential of chromium(VI) (Levina and Lay 2005; Reddy and Chinthamreddy 1999); and (2) the greater ability of chromium(VI) to enter cells (Costa 2003). Differences in molecular structure contribute the greater cellular uptake of chromium(VI) compared to chromium(III) (Costa 2003; Costa and Klein 2006a). At physiological pH, chromium(VI) exists as the tetrahedral chromate anion, resembling the forms of other natural anions (e.g., sulfate and phosphate) which are permeable across nonselective membrane channels. Chromium(III), however, forms octahedral complexes and cannot easily enter through these channels. Therefore, the lower toxicity to chromium(III) may be due in part to lack of penetration through cell membranes. It follows that extracellular reduction of chromium(VI) to chromium(III) may result in a decreased penetration of chromium into cells, and therefore, a decreased toxicity.

The higher redox potential of chromium(VI) contributes to the higher toxic potency of chromium(VI) relative to chromium(III) (Levina and Lay 2005), because once it is taken into cells, chromium(VI) is rapidly reduced to chromium(III), with chromium(V) and chromium(IV) as intermediates. These reactions commonly involve intracellular species, such as ascorbate, glutathione, or amino acids (Aiyar et al. 1991; Blankenship et al. 1997; Capellmann et al. 1995; Hojo and Satomi 1991; Kim and Yurkow 1996; Lin et al. 1992; Liu et al. 1997b; Mao et al. 1995; Wiegand et al. 1984; Zhitkovich et al. 1996). Chromium(VI), chromium(V), and chromium(IV) have all been shown to be involved in Fenton-like oxidative cycling, generating oxygen radical species (Aiyar et al. 1991; Chen et al. 1997; Liu et al. 1997b;

Luo et al. 1996; Mao et al. 1995; Molyneux and Davies 1995; Tsou et al. 1996). It is believed that the formation of these radicals may be responsible for many of the deleterious effects of chromium on cells, including lipid peroxidation (Bagchi et al. 2002a; Hojo et al. 1999, 2000) and alterations in cellular communication, signaling pathways and cytoskeleton (Chen et al. 1997; Gao et al. 2002; Gunaratnam and Grant 2002, 2004; Kim and Yurkow 1996; Mikalsen 1990; O'Hara et al. 2007; Shumilla et al. 1998; Wang et al. 1996a; Xu et al. 1996; Ye et al. 1995). Cellular damage from exposure to many chromium compounds can be blocked by radical scavengers, further strengthening the hypothesis that oxygen radicals play a key role in chromium toxicity (Hojo et al. 2000; Luo et al. 1996; Tsou et al. 1996; Ueno et al. 1995a).

The products of metabolic reduction of chromium(VI) (free radicals and chromium(IV) and (V)) and the newly generated chromium(III) are thought to be in part responsible for the carcinogenic effects seen in human and animal studies. The interaction of free radicals, chromium(V), chromium(IV), and chromium(III) with DNA can result in structural DNA damage, functional damage, and other cellular effects (Levina and Lay 2005; Singh et al. 1998a). The types of chromium-induced structural damage include DNA strand breaks (Aiyar et al. 1991; Bagchi et al. 2002a; Bryant et al. 2006; Casadevall et al. 1999; Ha et al. 2004; Kuykendall et al. 1996; Manning et al. 1992; Messer et al. 2006; Pattison et al. 2001; Ueno et al. 1995a), DNA-protein crosslinks (Aiyar et al. 1991; Blankenship et al. 1997; Capellmann et al. 1995; Costa et al. 1996, 1997; Kuykendall et al. 1996; Lin et al. 1992; Manning et al. 1992; Mattagajasingh and Misra 1996; Miller et al. 1991; O'Brien et al. 2005; Quievryn et al. 2001; Zhitkovich et al. 1996), DNA-DNA interstrand crosslinks (Xu et al. 1996), chromium-DNA adducts, and chromosomal aberrations (Blankenship et al. 1997; Sugiyama et al. 1986a; Umeda and Nishimura 1979; Wise et al. 1993). Functional damage includes DNA polymerase arrest (Bridgewater et al. 1994a, 1994b, 1998), RNA polymerase arrest, mutagenesis, and altered gene expression. However, DNA double strand breaks may not be due to free radical formation, but due to the formation of chromium-DNA ternary adducts, which lead to repair errors and collapsed replication forks (Ha et al. 2004). Double strand breaks can also lead to alterations in cellular communication and effects on signaling pathways and cytoskeleton. In addition, results of recent studies in human lung cells suggest that chromosome instability is an important mechansism in the development of lung cancers; specifically, chromium-induced chromosome instability appears to be mediated through centrosome and spindle assembly checkpoint bypass (Holmes et al. 2006; Wise et al. 2006a).

Location of particle deposition in the lung and extracellular dissolution of chromium(VI) compounds (e.g., solubility) are also important considerations regarding the mechansim of chromium(VI)-induced

carcinogenesis. In chromate workers, analysis of bronchial tissues shows higher chromium concentrations in areas of bronchial bifurcation compared to other areas in the bronchi (Ishikawa et al. 1994a). Also, autopsy results show that some precancerous bronchial lesions originated at bronchial bifurcations (Ishikawa et al. 1994b). Solubility of chromium(VI) compounds may also play a role in carcinogenic potency, with extracellular dissolution of the chromium compound critical to activity (Wise et al. 2004). This hypothesis is supported by *in vitro* data suggesting that extracellular chromium ions are the proximate clastogen in Chinese hamster ovary cells (Wise et al. 2004).

Chromium(III) can also interact with DNA to form adducts/complexes and DNA-protein crosslinks that interfere with DNA replication and transcription, and can promote the expression of regulatory genes such as nuclear factor-κβ, or may inhibit regulatory genes such as GRP78 (Chen et al. 1997; Kim and Yurkow 1996; Manning et al. 1992; Mikalsen 1990; O'Hara et al. 2003; Shumilla et al. 1998; Wang et al. 1996a; Xu et al. 1996; Ye et al. 1995). Disruption of these pathways by other compounds has been implicated in carcinogenesis. The structural and functional damage can lead to growth arrest (Xu et al. 1996) and apoptosis (Carlisle et al. 2000; Singh et al. 1999). Numerous studies show that chromium can induce apoptosis (Asatiani et al. 2004; Bagchi et al. 2001; Carlisle et al. 2000; Flores and Perez 1999; Gambelunghe et al. 2006; Gunaratnam and Grant 2002, 2004; He et al. 2007; Manygoats et al. 2002; Petit et al. 2004; Russo et al. 2005; Vasant et al. 2003); although the mechanism by which chromium induces apoptosis is not fully understood, it is believed to involve oxidative stress and activation of the p-53 protein (Pulido and Parrish 2003; Singh et al. 1998a).

3.5.3 Animal-to-Human Extrapolations

Species-related differences in chromium pharmacokinetics have been demonstrated, both between rodent species and between rodents and humans. However, studies directly examining species differences have been limited. Human microsomal chromium(VI) reduction is different from the P450-mediated microsomal reduction in rodents; specifically, the human system is much less oxygen-sensitive, has a much greater affinity for chromate, and is apparently mediated by flavoproteins (Myers and Myers 1998; Pratt and Myers 1993). Tissue distributions of chromium were found to be different between rats and mice after administration of bolus amounts of chromium(VI). Rat erythrocytes had a greater capacity to sequester chromium(VI) and reduce it to chromium(III) than mouse erythrocytes (Coogan et al. 1991b; Kargacin et al. 1993), thus demonstrating that both physiologic and metabolic differences can exist among species.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

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

Based on results of *in vivo* and *in vitro* studies, chromium(VI) may alter function of the hypothalamic-pituitary axis function. Serum prolactin levels were decreased by 59% in male Wistar rats exposed to 74 mg chromium(VI)/kg/day as potassium dichromate in drinking water for 30 days. Incubation of cultured rats anterior pituitary cells with 0.1–10 μM chromium(VI) as potassium dichromate decreased prolactin secretion and cell viability (Quinteros et al. 2007). No additional assessments of hypothalamic-

pituitary axis function were conduced in this study. Serum testosterone levels were decreased by 20.8% in male New Zealand rabbits administered 3.6 mg chromium(VI)/kg/day as potassium dichromate for 10 weeks by gavage (Yousef et al. 2006); however, since function of the hypothalamic-pituitary-gonadal axis was not assessed, it is unclear if this effect reflects endocrine disruption.

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and

sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990b; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

Chromium(III) is an essential nutrient required for maintaining normal glucose metabolism. The IOM of the NAS determined an adequate intake of 0.2 μg chromium/day for infants aged 0–6 months; 5.5 μg chromium/day for infants aged 7–12 months; 11 μg chromium/day for children aged 1–3 years; 15 μg chromium/day for children aged 4–8 years; 25 μg chromium/day for boys aged 9–13 years; 21 μg chromium/day for girls aged 9–13 years; 35 μg chromium/day for boys aged 14–18 years; and 24 μg chromium/day for girls aged 14–18 years (IOM 2001).

There is a limited amount of information available on the toxicity of chromium in children. Most of the available data come from several case reports of children ingesting lethal concentrations of chromium(VI). A variety of systemic effects were observed in a 22-month-old who accidentally ingested an unknown amount of sodium dichromate (Ellis et al. 1982), a 1-year-old who ingested an unknown amount of ammonium dichromate (Reichelderfer 1968), a 17-year-old who intentionally ingested 29 mg chromium(VI)/kg as potassium dichromate (Clochesy 1984; Iserson et al. 1983), and a 14-year-old who ingested 7.5 mg chromium(VI)/kg as potassium dichromate (Kaufman et al. 1970). The effects included pleural effusion, bronchopneumonia, hypoxic changes in the myocardium, decreased blood pressure and cardiac output, abdominal pain and vomiting, gastrointestinal burns and hemorrhage, and liver and kidney necrosis. An enlarged brain and cerebral edema were also observed in the 14-year-old (Kaufman et al.

1970). These effects are similar to effects observed in adults who have ingested lethal doses and are part of the sequelae leading to death.

A number of additional health effects have been observed in adults exposed to chromium (primarily chromium(VI)) at work. The primary targets appear to be the respiratory tract, gastrointestinal tract, hematological system, liver, and kidneys; an increased cancer risk has also been observed. Dermal contact in chromium sensitized individuals can lead to an allergic type dermatitis. In the absence of data to the contrary, it is likely that these organs/systems will also be sensitive targets in children. There is insufficient information to determine whether the susceptibility of children would differ from that of adults.

Although there are no human studies that examined developmental end points, animal studies have consistently shown that chromium, particularly chromium(VI), is a developmental toxicant. A number of developmental effects have been reported in oral studies involving maternal exposure to ≥46 mg chromium(VI)/kg/day as potassium dichromate (Al-Hamood et al. 1998; Junaid et al. 1996b; Trivedi et al. 1989). The observed effects included increases in postimplantation losses, gross abnormalities (e.g., subdermal hemorrhage, decreased ossification, kinky tail), and impaired development of the reproductive system (e.g., impaired fertility in female offspring). Similar developmental effects (e.g., post implantation losses, subdermal hemorrhage, decreased ossification) have also been observed in the offspring of rats and mice exposed to ≥37 mg chromium(VI)/kg/day for 20 or 90 days prior to mating (Junaid et al. 1996a; Kanojia et al. 1996, 1998). Conflicting results have been found for chromium(III). No developmental effects were reported in the offspring of rats fed 1,806 mg chromium(III)/kg/day as chromium oxide for 60 days before mating and throughout gestation (Ivankovic and Preussmann 1975). However, impaired development of the reproductive system (decreased reproductive tissue weight and impaired fertility) were observed in the offspring of mice exposed to 74 mg chromium(III)/kg/day as chromium chloride (Al-Hamood et al. 1998). Developmental effects have also been observed following intraperitoneal administration of chromium(III) chloride (Iijima et al. 1983; Matsumoto et al. 1976).

Chromium may be transferred to fetuses through the placenta and to infants via breast milk. Elevated levels of chromium have been reported in umbilical cord blood, placentae, and breast milk of women working in a dichromate(VI) manufacturing facility (Shmitova 1980). As noted elsewhere in the profile, the reliability of this study is suspect because the levels of chromium in the blood and urine of the control women were much higher than background levels. Measurement of the chromium content in 255 samples from 45 lactating American women revealed that most samples contained <0.4 μg/L, and the mean value

was 0.3 µg/L (Casey and Hambidge 1984). While these probably represent background levels in women whose main exposure to chromium is via the diet, the findings indicate that chromium may be transferred to infants via breast milk. These findings in humans are supported by animal data. Studies in rats and mice have shown that chromium(VI) and chromium(III) crosses the placenta and enters into fetal tissue. Elevated levels of chromium have been observed in the placenta and fetal tissue of rats and mice exposed to potassium dichromate(VI) in drinking water during pregnancy (Saxena et al. 1990a). The levels of chromium in the placenta were 3-and 3.2-fold higher in the exposed rats and mice, respectively, than in controls and fetal tissue chromium levels were 3.1-and 9.6-fold higher, respectively; the difference over control was only statistically significant in the mice. Another study (Danielsson et al. 1982) also found elevated fetal tissue levels of chromium. The chromium levels in the fetal tissues were 12–19% of maternal blood levels following maternal intravenous injections of sodium dichromate(VI) on gestational days 12–15 or 16–18 and 0.4–0.8% following maternal intravenous injections of chromium(III) trichloride on gestational days 12–15 or 16–18. A study of transplacental transfer of chromium(III) in different forms indicated that placental transport varies with the chemical form (Mertz et al. 1969). Higher levels of chromium were found in the neonates of rats fed chromium in a commercial diet as compared to neonates of rats fed a chromium-deficient diet and given drinking water containing chromium acetate monohydrate. Similarly chromium levels were significantly elevated in the offspring of rats administered chromium in the form of chromodulin from Brewer's yeast by gavage than in the offspring of rats administered chromium trichloride intravenously or by gavage.

There is very little information available in which to assess whether the pharmacokinetic properties of chromium would be different in children. Sullivan et al. (1984) found that gastrointestinal absorption of radiolabeled chromium chloride, administered by gavage, was 10 times higher in 2-day-old rats as compared to levels absorbed in adult rats. A similar pattern of distribution in the body was found in the immature and mature rats.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment

of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to chromium are discussed in Section 3.8.1.

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

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

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Chromium

As an essential nutrient, chromium is normally present in blood and urine. Chromium in body fluids (e.g., blood and urine) is the exposure biomarker of choice. Mean dietary chromium intake in the general U.S. population was estimated as 0.505 μg/kg/day (equivalent to 35.35 μg/day, assuming a body weight of 70 kg), with a range of 0.293–0.867 μg/kg/day (Moschandreas et al. 2002); however, only a small amount of dietary chromium is absorbed (≤3%). The IOM of the NAS (IOM 2001) determined an

adequate intake (e.g., a level typically consumed by healthy individuals) of 20–45 μg chromium(III)/day for adolescents and adults. Daily dietary intake levels have been shown to correlate with total excretion of chromium in the urine and feces (Bunker et al. 1984). The IOM (2001) reported average plasma chromium concentrations of 2–3 nmol/L (equivalent to 0.10–16 μg/L) and an average urinary chromium excretion of 0.22 μg/L or 0.2 μg/day; endogenous chromium concentrations also have been reported as 0.01–0.17 μg/L (median 0.06 μg/L) in serum (Sunderman et al. 1989), 0.24–1.8 μg/L (median 0.4 μg/L) in urine (Iyengar and Woittiez 1988), and 0.234 mg/kg in hair (Takagi et al. 1986). However, normal chromium levels in human fluid and tissues should be interpreted with caution. The low sensitivity of the most commonly used detection methods and the ubiquitous presence of chromium in laboratories make detection of low levels of chromium in blood and urine difficult.

Exposure to chromium may result in increased chromium concentrations in blood (whole blood, serum, and erythrocytes), urine, expired air, hair, and nails; of these, elevations of chromium in blood and urine are considered the most reliable indicators of exposure (Barceloux 1999; Caglieri et al. 2006). Urinary elimination half-times for absorbed chromium(III) range from 10–40 hours (Kerger et al. 1996a). Assuming an elimination half-time of 40 hours, steady state, plasma concentration, and urinary excretion rate of chromium would reach 95% of steady state levels in approximately 7 days (Paustenbach et al. 1996). Once steady state is achieved, the daily amount of chromium excreted in urine will reflect the daily amount absorbed. With cessation of exposure levels of chromium in plasma and urine will reach 5% of steady state within 7 days. The relatively rapid elimination kinetics of absorbed chromium(III) has implications for the use of plasma and urine as biomarkers of exposure to chromium. Plasma and urinary chromium concentrations will largely reflect relatively recent exposure (i.e., exposures that occurred several weeks prior to the sample may not be detected from plasma or urinary chromium measurements). During relatively constant or repetitive exposures that achieve a steady state in plasma, daily urinary chromium excretion measured on a single day can be expected to be highly correlated with chromium intake. This correlation will weaken with greater intermittency in the exposure, with greater dependence on the time of sampling with respect to the most recent exposure. The above general principles apply to exposures to absorbed chromium(III) compounds; however, absorbed chromium(VI) has a longer retention time in blood. Chromium(VI) that enters blood is taken up by red blood cells, reduced to chromium(III), and, in the process, form adducts with red blood cell hemoglobin and other proteins. These complexes are sufficiently stable to remain in the red blood cells for a substantial fraction of the lifespan of the red blood cell. Therefore, following absorption of chromium(VI) in to blood, the elimination half-time of chromium in blood will be substantially longer than that in plasma. The elimination half-time of injected chromium(VI) (e.g., sodium chromate-51, used in the clinical assessment

of red blood cell volume) is approximately 25–35 days (Dever et al. 1989). Based on a half-time of 30 days in red blood cells, with cessation of exposure to and absorption of chromium(IV), levels of chromium in red blood cells will reach 5% of a previous steady state level within 130 days.

Although chromium also accumulates in white blood cells, erythrocyte chromium has been shown to be a more sensitive measure of chromium exposure (Coogan et al. 1991b; Lukanova et al. 1996). An increase in plasma levels of chromium may reflect both recent exposure and exposure that occurred during the past few months (e.g., chromium that is sequestered within erythrocytes for the lifespan of the cell), whereas elevated urine chromium primarily reflects exposure over the past 1–2 days (Barceloux 1999). Distinct measurements of chromium in plasma and whole blood (reflecting intracellular distribution to erythrocytes) also be useful in distinguishing exposures to chromium(VI) compounds versus chromium(III) compounds; increased plasma levels of chromium may indicate exposure to both chromium(VI) and chromium(III), whereas increased chromium in erythrocytes indicates exposure to chromium(VI), since chromium(III) is not taken up by erythrocytes. For example, evaluation of postshift whole blood, serum, erythrocytes, and urine in groups of dichromate production workers exposed mainly to chromium(VI) or chromium(III) showed relationships between exposure type (e.g., chromium(VI) or chromium(III)) and chromium in blood and urine (Minoia and Cavalleri 1988). In 22 workers exposed primarily to chromium(VI) (0.008–0.212 mg chromium(VI)/m^3, 0.010–0.10 mg chromium(III)/m^3), the mean postwork-shift urinary chromium level was 31.5 μg total chromium/L; chromium(VI) was not detected in the urine samples (detection limit=0.05 μg chromium(VI)/L) due to *in vivo* reduction of chromium(VI) to chromium(III). Concentrations of total chromium in serum, erythrocytes, and whole blood were 2.2, 8.9 and 6.9 μg/L, respectively; compared with control levels of 1.1, 1.0, and 1.4 μg/L, respectively. In 15 workers exposed primarily to chromium(III) (0.046–1.689 mg chromium(III)/m^3, 0.002–0.023 mg chromium(VI)/m^3), the mean postwork-shift urinary chromium level was 24.7 μg total chromium/L and concentrations of total chromium in serum, erythrocytes, and whole blood were 3.1 μg/L, 1.4, and 1.8, respectively. The level of chromium in serum of the workers exposed mainly to chromium(III) was significantly ($p<0.001$) higher than that measured in workers exposed mainly to chromium(VI) or in controls. The level of chromium in erythrocytes of the workers exposed mainly to chromium(III) was significantly ($p<0.001$) less than that in workers exposed mainly to chromium(VI). The finding of higher levels of chromium in serum and lower levels of chromium in erythrocytes of workers exposed mainly to chromium(III) than in workers exposed mainly to chromium(VI) reflects the relative inability of chromium(III) to enter erythrocytes.

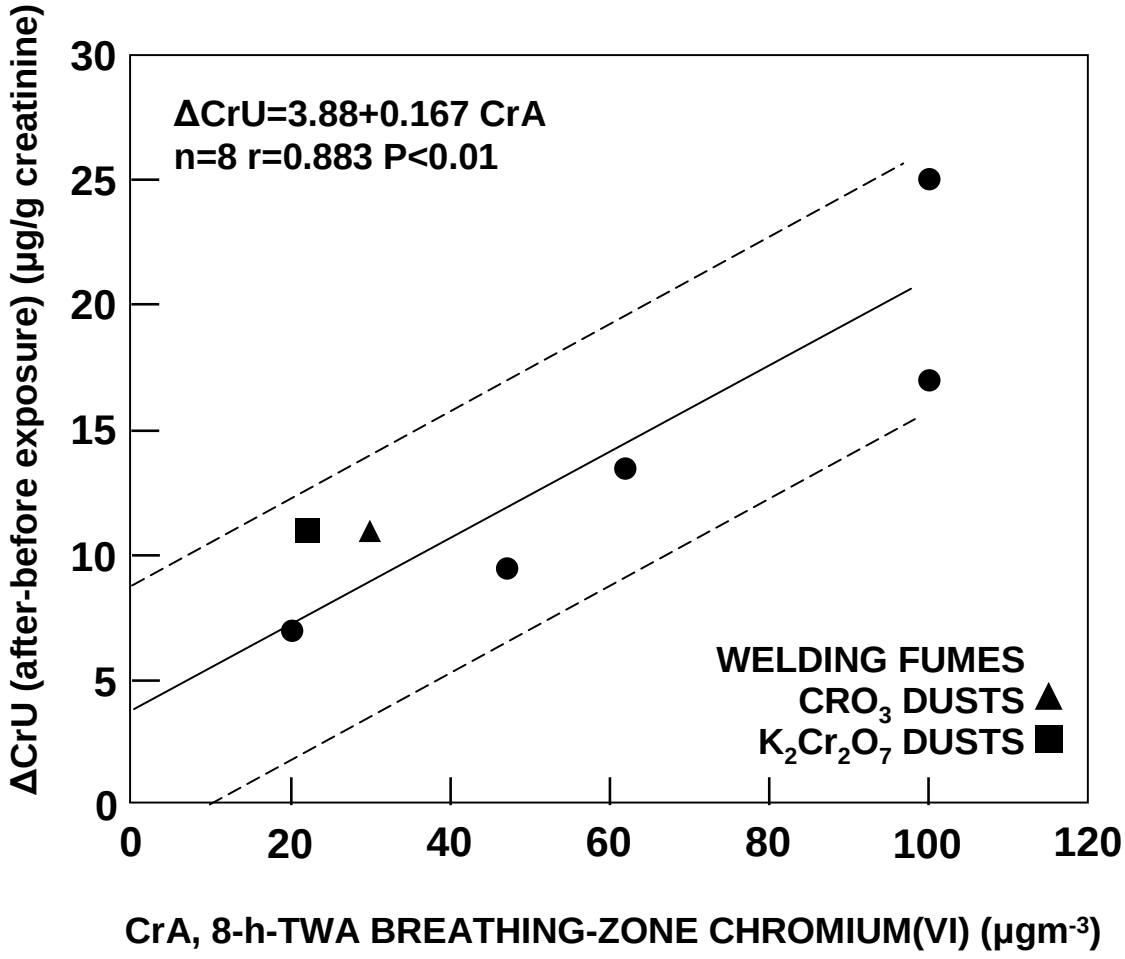
Although exposure to chromium may produce increases in chromium levels in both blood and urine chromium levels, the relationship between blood and urinary chromium levels may vary. Entry of chromium(VI) into the red blood cells probably reflects a competition between plasma reduction to chromium(III) and red blood cells uptake of chromium(VI) and not the result or consequence of the exhaustion of plasma reducing ability. When hexavalent chromium is incubated with washed isolated erythrocytes, almost all of the entire dose is taken up by the cells. Chromium(VI) is then reduced inside the cells to trivalent chromium, essentially trapping it inside the erythrocyte. In contrast, little chromium(III) appears to be taken up by erythrocytes *in vitro* incubations (Aaseth et al. 1982; Bentley 1977; Donaldson and Barreras 1966; Gray and Sterling, 1950). When chromium(VI) is incubated with whole blood or erythrocytes plus plasma, only a fraction (depending on conditions) of the chromium(VI) is taken up by the erythrocytes (Coogan et al. 1991b; Corbett et al. 1998; Lewalter et al. 1985; Wiegand et al. 1985), most likely due to the reduction of a portion of chromium(VI) to chromium(III) outside of the erythrocyte (Capellmann and Bolt 1992; Korallus et al. 1984; Richelmi et al. 1984). Thus, chromium(III) is then largely excluded from the erythrocyte. However, Korallus (1986a, 1986b) has proposed that the relationship between blood and urinary chromium levels may vary, possibly due to variability in plasma reduction capacity. *In vitro* experiments indicate that when chromium(VI) plasma levels exceed the plasma reduction capacity (PRC), chromium(VI) enters erythrocytes, is reduced, and binds to hemoglobin. The bond persists for the lifetime of the erythrocytes (120 days); therefore, a single determination of chromium in erythrocytes allows a longitudinal evaluation of exposure for an extended period in the past. Low chromium concentrations in erythrocytes indicate that the amount of chromium(VI) uptake did not exceed the PRC. Limited evidence suggests that the capacity of human plasma to reduce chromium(VI) compounds to chromium(III) compounds varies, with slow and fast reducers recognized (Korallus 1986a, 1986b). It is not clear what is responsible for individual differences in the PRC, although difference in magnitude of PRC appears to correlate with the levels of ascorbic acid in plasma.

The relationship between serum and urine chromium levels to occupational exposure levels has been investigated in numerous studies, with results showing correlations between exposure levels and chromium levels in blood and urine (Gylseth et al. 1977; Iarmarcovai et al. 2005; Kilburn et al. 1990; Lewalter et al. 1985; Lindberg and Vesterberg 1983a; McAughey et al. 1988; Medeiros et al. 2003a; Minoia and Cavalleri 1988; Muttamara and Leong 2004; Mutti et al. 1985b; Randall and Gibson 1987, 1989; Saner et al. 1984; Sathwara et al. 2007; Simpson and Gibson 1992; Sjogren et al. 1983; Stridsklev et al. 2004; Takagi et al. 1986; Tola et al. 1977; Wiegand et al. 1988). In workers exposed to chromium(VI) as chromium trioxide in the chrome plating industry, a significant correlation (r=0.71) was

observed found between exposure levels and postshift urinary chromium; for a TWA exposure of 0.002 mg chromium(VI)/m^3, the mean urinary chromium level was 5.2 μg/L (excluding workers with obvious skin contamination) (Lindberg and Vesterberg 1983a). Significant correlations were observed between chromium concentrations in air (measured by personal sampling devices; 8-hour TWA) and levels of chromium in blood (r=0.99) and urine (r=0.89) in workers at a chromium alloy facility (Muttamara and Leong 2004). In areas of low exposure, the air concentration of chromium (type not specified) was 5.75 μg/m^3; in workers in this area, mean chromium concentrations in blood and urine (duration of sample collection was not reported) were 0.925 and 0.095 μg/dL, respectively. In areas of high exposure, the air concentration of chromium was 7.25 μg/m^3, in workers in this area, mean chromium concentrations in blood and urine were 3.64 and 0.34 μg/dL, respectively. An increase in urinary chromium of 12.2 μg/g creatinine above preexposure values or a total concentration of 29.8 μg/g creatinine (end-of-shift values) corresponded to an air concentration of 50 μg chromium(VI)/m^3 from welding fumes (Mutti et al. 1985b). Examination of end-of-shift chromium levels indicated a correlation between urinary chromium levels and exposure to soluble chromium(VI) compounds, but not to insoluble chromates or chromium(III) compounds (Minoia and Cavalleri 1988; Mutti et al. 1985b). The relationship between workroom air concentrations of water soluble chromium(VI) compounds and daily increases in urinary chromium (preexposure values subtracted from end-of-shift values) are shown in Figure 3-7. Serum and urine concentrations of chromium were significantly elevated in a group of 73 tannery workers, with exposure primarily to chromium(III) compounds, compared to a group of 52 control subjects, at the end of the workweek on Friday and before exposure began on Monday (Randall and Gibson 1987). Serum and urine chromium levels correlated with work area of the tannery, with the highest concentrations in workers handling wet hides in the chrome tanning and wringing departments. The time-weighted average level of total chromium in tannery air was 1.7 μg/m^3 and did not vary significantly among the various tanneries involved in the study or among the various work areas of each tannery, with chromium(VI) levels in tannery air were below the detection limit of (0.1 μg/m^3).

Urinary and blood chromium have also been used as a biomarker for environmental exposure (Bukowski et al. 1991; Chang et al. 2006; Fagliano et al. 1997). However, interpretation of results may be limited by several factors, including that exposure must be sufficient such that urinary and blood concentrations are higher than the range of background concentrations and analytical limit of detection, high inter- and intrapersonal variability, and that different chemical forms have different bioavailabilities (Paustenbach et al. 1997; Finley et al. 1996b; Gargas et al. 1994; Kerger et al. 1997). Furthermore, the short half-life of chromium (e.g., at least 90% of absorbed chromium is eliminated within 24 hours) make it difficult to assess exposure incidents. Low-level, intermittent exposure, such as would occur with environmental

Figure 3-7. Relationship Between Water Soluble Chromium(VI) CrA and Daily Increase in Urinary Chromium Levels (CrU) (Pre-exposure Values were Subtracted from End-of-Shift Values)



Source: Mutti et al. 1985b

exposures to soil, dust, and residential drinking water, may not be detected with urinary monitoring; however, it is more likely that urinary monitoring would detect higher-level continuous exposure or daily inhalation exposure to chromium(VI). Paustenbach et al. (1997) note that chromium intakes would have to exceed 2 μg/day in order to distinguish the exposure from background. Large interpersonal variability (as high as a factor of 10) and intrapersonal variability (as high as a factor of 3) can result in highly variable erroneous conclusions regarding significant differences among populations.

3.8.2 Biomarkers Used to Characterize Effects Caused by Chromium

Chromium has been shown to produce effects to several systems, including the respiratory, gastrointestinal, hematological, and immunological systems (see Section 3.2); however, many of these effects are not specific for chromium. Although effects to these physiological systems can be assessed with blood and respiratory function tests and by physical examination, these assessments would not serve as biomarkers specific for effects of chromium as impairment of these systems can result from a variety of other causes, including chemical toxicity, nutritional insufficiencies, and disease.

Occupational exposure to chromium and its compounds has caused respiratory effects, such as pneumonitis, impaired pulmonary function, nasal septum perforations, irritation of the mucosa, inflammation, and cancer. In addition, chromium can be irritating and corrosive to the skin. Chromium exposure may cause asthma attacks and dermatitis in sensitive individuals. Workers with urinary levels of chromium >15 μg/g creatinine had increased retinol binding protein and tubular antigens in the urine. The workroom levels ranged from 0.05 to 1.0 mg chromium(VI)/m^3 as chromium trioxide (Franchini and Mutti 1988). The urine of chromium(VI) exposed workers in a chromate production plant contained higher levels of a brush border protein and of retinol-binding protein in the urine than did nonexposed controls (Mutti et al. 1985a). In a study of currently exposed chrome platers, ex-chrome platers, and referent groups of nonexposed workers, the urinary levels of β_2-microglobulin were significantly higher ($p=0.045$), and elevated levels occurred more often in the presently exposed groups compared with its age-matched control group. The levels of β_2-microglobulin in the urine of the ex-chrome platers, however, were not different than those of its age-matched control group (Lindberg and Vesterberg 1983b). Another study of hard chrome electroplaters found a higher prevalence of workers with elevated N-acetyl-β-glucosamindase levels (Liu et al. 1998). Although this study also found higher levels of β_2-microglobulins in the chrome plater, the prevalence of elevated values was not significantly increased. The presence of low molecular weight proteins, such as retinol binding protein, antigens, or

$ß_2$-microglobulin in the urine is believed to be an early indication of kidney dysfunction. The lack of a significant difference in the ex-chrome platers compared with the control group suggests that the chromium-induced kidney damage may be reversible. Cell culture and cell free studies discussed in Section 3.5.2 demonstrated that chromium forms protein-DNA crosslinks and adducts with DNA, and that these end points may be potentially useful biological markers, indicating the possibility of genotoxic effects or cancer in humans exposed to chromium. However, no increases in protein-DNA crosslinks were observed in white cells from volunteers who were exposed to chromium(VI) in drinking water (Kuykendall et al. 1996).

The possibility of using an immune-function assay as a potential biomarker for humans exposed to chromium has been examined (Snyder et al. 1996). Isolated mononuclear cells from 46 individuals who lived and/or worked in areas in northern New Jersey at sites contaminated by chromium processing were stimulated by pokeweed mitogen. Rates of stimulated cell growth and production of interleukin 6 (IL-6) were measured and compared to a control population of people who lived/worked in uncontaminated areas. There was no significant increase in mitogen stimulation between people from contaminated areas and controls, but there was a significant (36%) decrease in the levels of IL-6 in monocytes in the chromium exposed group. IL-6 is an important cytokine that is involved in the T-cell helper pathway of antibody production. The significance of the lower levels may lead to decreased levels of antibody production.

The effects of chromium(III) chloride, sodium chromate(VI), and potassium chromate(VI) on proliferation of mononuclear leukocytes obtained from chromium sensitive individuals (confirmed with positive patch tests) was compared to nonsensitive controls (confirmed by negative patch tests) (Räsänen et al. 1991). Isolated cells were exposed to 25–50 µg/mL culture medium of chromium(III) chloride and to 0.025 to 0.1 µg/mL culture medium chromium(VI) salts, which gave optimum responses and cell growth ratios of treated/nontreated cells from eight sensitive individuals ranging from 1.56 to 13.22, average=5.8 (chromium(III)), from 2.24 to 11.43, average 5.4 sodium chromate, and from 1.82 to 9.48, average 5.4potassiium dichromate. The nonsensitive individuals' ratios were consistently lower with ranges from 0.90 to 2.28 and average ratios of 1.14, 1.30, and 1.56, respectively. The authors felt that this *in vitro* methodology could be used diagnostically to assess chromium-sensitive individuals.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (Agency for Toxic Substances and Disease Registry 1990c) and for information on biomarkers for neurological effects see OTA (1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Potassium dichromate (10 mg/kg) administered by subcutaneous injection potentiated the effects of the nephrotoxins, mercuric chloride, citrinin, and hexachloro-1,3-butadiene, in rats. Effects on renal function included changes in urine volume, osmolality, electrolyte and glucose excretion, and a reduction in renal cortical slice organic ion transport. Chromium(VI) compounds potentiated the effect of mercuric chloride on organic acid uptake but not organic base uptake by renal cortical slices (Baggett 1986; Haberman et al. 1987). A similar experiment with another nephrotoxin, maleic acid, demonstrated the potentiating effect of potassium dichromate (10 mg/kg administered subcutaneously) (Christenson et al. 1989). Christenson et al. (1989) suggested that the combination of potassium dichromate with maleic acid might enhance damage to the brush border of the renal proximal tubules or that damage to the luminal cells by potassium dichromate might allow maleic acid to more easily enter the cells.

Concomitant exposure of female Sprague-Dawley rats to chromium(VI) potassium dichromate and ethanol in drinking water for 22 weeks indicates that ethanol may enhance the hepatic effects of chromium(VI) (Acharya et al. 2001). Serum enzyme activity of ALT in rats treated with 10% ethanol and 25 mg chromium(VI)/L (3.8 mg chromium(VI)/kg/day) was significantly increased compared to treatment of rats with ethanol or chromium(VI) alone. However, the toxicological significance of this finding is uncertain, since serum ALT activities of rats treated with ethanol and chromium(VI) were increased by only 18% compared to treatment of rats with chromium(VI) alone.

Interactions between selenium in the diet and ammonium dichromate in the drinking water were investigated in a study using rats. During the experiment, one rat died and the other rats had atrophy of the central liver lobe when given selenium alone. Dietary selenium and ammonium chromate in combination caused hepatic necrosis, resulting in the death of four rats (Moxon and DuBois 1939). Although the rats were not fed chromium alone, other studies indicate that the liver is a target for chromium exposure (see Section 3.2). The mechanism for the interaction was not discussed.

Exposure of female hairless mice to ultraviolet light in combination with chromium(VI) as potassium chromate in drinking water at concentrations of 2.5 or 5.0 mg potassium chromate(VI)/L (approximately 0.18 or 0.35 mg chromium(VI)/kg/day) for 182 days, or in the diet at concentrations of 0, 2.5, or 5.0 mg potassium chromate(VI)/kg food (approximately 0.13 or 0.26 mg chromium(VI)/kg/day) for 26 weeks, produced an increased incidence of skin tumors compared to animals exposed to UV light alone or

chromium(VI) alone (Davidson et al. 2004; Uddin et al. 2007). Exposure to chromium(VI) alone did not result in neoplasms. The chromium-induced enhancement of UV light-induced skin tumors did not appear to be mediated through oxidative damage, since concomitant treatment with vitamin E or selenomethionine did not decrease the chromium effect.

Concomitant inhalation exposure to ozone and chromium(VI) may alter pulmonary clearance mechanisms in the deep lung (Cohen et al. 2003). Compared to rats treated with insoluble chromium(VI) as calcium chromate (0.34–0.36 mg chromium(VI)/m^3) alone for up to 48 weeks, concomitant exposure to ozone (0.59 mg/m^3) resulted in decreased particle uptake and altered postphagocytic/endocytic processing of chromium particles by alveolar macrophages. However, since toxicity was not assessed in this study, it is not known if ozone-induced alteration in alveolar macrophage function would result in increased toxicity of chromium(VI).

A number of studies indicate an increase in the mutagenic effects of chromium(VI) compounds in combination with other chemicals. Synergism has been observed between chromium(VI) and 9-aminoacridine, nitrilotriacetic acid, and azide (Bronzetti and Galli 1989; Gava et al. 1989a; LaVelle 1986a, 1986b; Montaldi et al. 1987), but the mechanisms are not clearly understood. Potassium dichromate potentiated mutations produced by sodium azide in *S. typhimurium* or by 9-aminoacridine in *S. typhimurium* and *E. coli*. Although the data were insufficient for speculation on the specific biochemical mechanism, it was suggested that the potentiation involved a specific effect of potassium dichromate on the interaction of 9-aminoacridine or sodium azide with DNA or on subsequent DNA replication and/or repair (LaVelle 1986a, 1986b). Nitrilotriacetic acid, which appears to have no genotoxic potential itself, increased the frequencies of sister chromatid exchanges in Chinese hamster ovary cells and of micronuclei and chromosomal aberrations in cultured human lymphocytes that were seen with lead chromate alone. However, nitrilotriacetic acid had no effect on the dose-related induction of sister chromatid exchanges in Chinese hamster ovary cells that was seen with potassium chromate alone. It was suggested that nitrilotriacetic acid increased the solubility of the originally insoluble lead chromate, leading to increased uptake of the metal cation by the cells and subsequent increased genotoxicity (Montaldi et al. 1987). Nitrilotriacetic acid increased the frequency of point mutations in *S. cerevisiae* observed with a low concentration of sodium chromate, but decreased the frequency with a 5-fold higher concentration of sodium chromate. It was suggested that at the low concentration of sodium chromate, nitrilotriacetic acid affected the uptake of chromium(VI), favoring reduction to chromium(III) ions, which formed a complex with nitrilotriacetic acid that can cross the membrane and interact with DNA. At the high dose of sodium chromate, nitrilotriacetic acid may have affected the mechanism of

recombination repair of DNA breaks induced by chromate oxidizing activity (Bronzetti and Galli 1989). Nitrilotriacetic acid also increased the mutagenicity of potassium dichromate in *S. typhimurium* and *D. melanogaster*, presumably by favoring the reduction of chromium(VI) to chromium(III) (Gava et al. 1989a). Thus, it is possible that other hazardous substances at hazardous waste sites may be more dangerous due to the presence of chromium(VI).

Ascorbic acid has been shown to have a protective effect in rats administered lethal dermal doses of potassium dichromate (25 mg chromium(VI)/rat), and in preventing ulcerations of the skin (Samitz 1970). The nephrotoxicity due to subcutaneous injections of potassium chromate in rats was prevented by intramuscular administration of ascorbic acid (Powers et al. 1986). This occurred mainly through the reduction of chromium(VI) to the less toxic chromium(III) state. In cultured human bronchial cells, co-exposure to ascorbic acid and sodium chromate blocked chromate-induced clastenogenicity by preventing uptake of chromium(VI) ions by cells (Wise et al. 2004). Vitamin E protected against, while vitamin B_2 enhanced, the cytotoxicity and DNA strand breaks induced by sodium chromate in Chinese hamster cells *in vitro*. Vitamin E may exert its protective effect by scavenging radicals and/or chromium(V) during the reduction of chromium(VI) (Sugiyama 1991) (see Section 3.11.3). N-Acetylcysteine, the glutathione precursor, was reported to be effective in increasing the urinary excretion of chromium in rats (Nadig 1994).

Studies have examined the effects of interactions between chromium and arsenic on blood cholesterol and glucose levels and changes in organ weight in rats (Aguilar et al. 1997). Groups of five male Wistar rats were given food containing 5 μg/g of either arsenic(V) oxide, chromium(III) chloride, or a combination of both chemicals for 10 weeks. Organ weight to body weight ratios of liver, spleen, lung, kidney, and heart were similar to control values for the three exposed groups. Arsenic alone increased the cholesterol blood level from 47.27(±6.85 SD) mg/dL in the control group to 96.83(±6.11 SD). The combination of arsenic and chromium reduced the blood cholesterol level to 46.69(±6.11 SD) mg/dL. Neither chemical alone or in combination affected blood glucose levels. In most tissues, the combination of chemicals reduced the chromium level appreciably below control values. Supplemental chromium increased arsenic levels in liver, kidney, spleen, heart, and red blood cells, and reduced levels of arsenic in lung and hair tissues. Chromium did not appear to alter concentrations of arsenic in the liver.

A study examining the chromium(VI) reduction in microsomes noted that the level of iron in the test system markedly influenced the V_{max} of chromium(VI) reduction, suggesting that coexposure to

chromium(VI) and agents that increase intracellular iron might lead to increased risk for chromium(VI) toxicity (Myers and Myers 1998).

The effects of chromium(III) chloride and sodium chromate(VI) on the hepatotoxicity of carbon tetrachloride exposure to mouse hepatocytes were examined by Tezuka et al. (1995). Primary cultures of mouse hepatocytes were pretreated with 10 or 100 μM chromium for 24 hours followed by exposure to 1–5 mM carbon tetrachloride for up to 1 hour. Chromium(VI) pretreatment significantly reduced the cell toxicity as well as lipid peroxidation caused by carbon tetrachloride. Chromium(III) pretreatment did not have any effect on cell toxicity. About 50% of chromium(VI) was taken up and reduced in the cells by 90% to chromium(III) within 10 minutes. The initial uptake rate of chromium(III) into cells was greater than 500-fold less than chromium(VI), and only about 5% was absorbed. The protection against carbon tetrachloride damage by chromium(VI) was attributed to its rapid uptake and conversion to chromium(III), and it was determined that chromium(III) acts as a radical scavenger for the free radicals generated by carbon tetrachloride within the cell. Furthermore, chromium(VI) pretreatment reduced the activity of NADPH cytochrome c reductase, which metabolizes carbon tetrachloride to reactive species. In a previous study (Tezuka et al. 1991), the same group found that pretreating mice and rats with chromium(III) also protected against hepatic toxicity.

In order to examine the speciation of chromium in lemonade, Kool Aid, tea, dripped coffee, percolated coffee, and orange juice, potassium chromate(VI) was added to each of the beverages at a chromium concentration of 10 mg/L (Kerger et al. 1996b). After 15 minutes, the concentrations of chromium(VI) were determined to be <0.4 mg/L for orange juice, <0.3 mg/L for coffee and tea, 2 mg/L for Kool Aid, and 0.3 mg/L for lemonade. After 3–5 hours, essentially no residual chromium(VI) remained. At higher concentrations (50 mg/L chromium(VI)), >99, 40, and 84% of the chromium(VI) was reduced after 3–5 hours in orange juice, lemonade, and coffee, respectively (not tested at the higher concentration in Kool Aid and tea). The reducing capacities were not correlated with total organic carbon or pH. The reducing capacities of the beverages were attributed in part to ascorbic acid in lemonade and orange juice and to tannins in tea and coffee.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chromium than will most persons exposed to the same level of chromium in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke).

These parameters result in reduced detoxification or excretion of chromium, or compromised function of organs affected by chromium. Populations who are at greater risk due to their unusually high exposure to chromium are discussed in Section 6.7, Populations with Potentially High Exposures.

Acute inhalation LC_{50} and oral and dermal LD_{50} studies suggest that female animals are more sensitive to the lethal effects of chromium(VI) compounds (see Sections 3.2.1.1, 3.2.2.1, and 3.2.3.1). Whether human females are more sensitive than males to toxic effects of chromium or its compounds is not known. Other information identifying possible susceptible populations was not located. The primary and most sensitive effects of exposure to chromium compounds to the respiratory, gastrointestinal, hematological, and immunological systems; thus, individuals with preexisting conditions of these systems may be at increased risk of exposure to chromium compounds. Due to the sensitizing effects of chromium, some individuals who are sensitive to chromium may develop asthma as an anaphylactic response to inhaled chromium. Also, there is limited evidence in some individuals have less ability than others to reduce chromium(VI) in the bloodstream and are more likely to be affected by the adverse effects of chromium exposure (Korallus 1986a, 1986b). The ability to reduce chromium(VI) in the bloodstream may be related to the ascorbic levels in the plasma. However, the metabolic reduction of chromium(VI) may result in bioactivation and/or detoxification.

Since chronic inhalation of cigarette smoke may result in squamous metaplasia in the respiratory mucosa, the risk of lung cancer due to inhalation of carcinogenic chromium compounds may be exacerbated in individuals who smoke cigarettes or are excessively exposed to passive smoke (Albert 1991).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

Haddad LM, Shannon MW, Winchester JF, eds. 1998. Chromium. In: Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B. Sanders Company, 794-795.

Leikin JB, Paloucek FP, eds. 2002. In: Leikin and Paloucek's poisoning and toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 372-379.

Schonwald S. 2004. Chromium. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippicott Williams & Wilkins, 1415-1417.

3.11.1 Reducing Peak Absorption Following Exposure

General recommendations for reducing absorption of chromium following acute inhalation exposure have included moving the patient to fresh air, monitoring for respiratory distress, and administering humidified supplemental oxygen with assisted ventilation if required (Haddad et al. 1998; Schonwald 2004). If pulmonary effects such as bronchoconstriction are present, treatment with oxygen and bronchodilator drugs may be administered (Haddad et al. 1998). The absorption of inhaled chromium compounds depends on such factors as oxidation state, particle size, and solubility. Chromium(VI) passes through the alveolar lining of the lungs to the bloodstream more readily than does chromium(III) (see Section 3.4.1.1), and more soluble compounds are absorbed more readily than those that are less soluble (Bragt and van Dura 1983). Although chromium(VI) is more readily absorbed from the lungs than chromium(III), various components of the respiratory system can reduce chromium(VI) to chromium(III), which is far less capable of crossing cell membranes than chromium(VI), thereby reducing the bioavailability of chromium to target cells other than the lung (De Flora and Wetterhahn 1989). Epithelial lining fluid (ELF) is capable of reducing chromium(VI) (Petrilli et al. 1986b) and may represent the first line of defense against inhaled chromium(VI). Ascorbic acid (vitamin C) and glutathione, both of which were found to reduce chromium(VI) to chromium(III) in cell-free bronchoalveolar lavage fluid or soluble fractions of rat lungs *in vitro*, appear to be involved in this activity of ELF (Suzuki and Fukuda 1990). Uptake and reduction of chromium(VI) by pulmonary alveolar macrophages, catalyzed by NADH- or NADPH-dependent cytosolic enzyme activities, may lead to virtually irreversible sequestration and efficient removal by mucociliary action (De Flora and Wetterhahn 1989; De Flora et al. 1984, 1987b). Reduction of chromium(VI) within pulmonary alveolar macrophage homogenates was stimulated in rats by the administration of the glutathione precursor, N-acetylcysteine (De Flora and Wetterhahn 1989). As mentioned above, the reduction of chromium(VI) to chromium(III) by these various processes within the lungs serves as a natural defense mechanism by decreasing the amount of chromium absorbed and enhancing mucociliary clearance of chromium(III). However, reduction of chromium(VI) to chromium(III) generates reactive intermediates, which may produce adverse effects. Theoretically, further clearance from the lungs might be achieved by the administration of expectorants, but the efficacy of such a procedure has not been tested.

Chromium(III) is also poorly absorbed by the gastrointestinal tract, and chromium(VI) is reduced to chromium(III) in the gastric environment, limiting the bioavailability of chromium(VI) (De Flora et al. 1987a; Donaldson and Barreras 1966). Thus, the oral toxicity of chromium metal is low. However, chromium(VI) compounds are highly corrosive to the gastrointestinal tract and can lead to hepatic, renal, hematological, and neurological effects (Clochesy 1984; Coogan et al. 1991a; Diaz-Mayans et al. 1986; Iserson et al. 1983; Kaufman et al. 1970; Kumar and Rana 1982, 1984; Samitz 1970; Saryan and Reedy 1988). The reduction of chromium(VI) to chromium(III) in the stomach is greatly enhanced at low pH and shortly after meals due to increased gastric juice secretion (De Flora et al. 1987a). Therefore, administration of food might help decrease the gastrointestinal absorption of chromium. The enhanced reduction of chromium(VI) at low pH suggests that, theoretically, oral administration of bicarbonates and antacids should be avoided. Oral administration of ascorbic acid to further reduce chromium(VI) to chromium(III) might further decrease bioavailability (Haddad et al. 1998; Schonwald 2004), although this has not been proven (Leikin and Paloucek 2002; Schonwald 2004). Other recommendations for reducing gastrointestinal absorption of chromium include diluting with water or saline followed by gastric lavage (Schonwald 2004). Inducing emesis with syrup of ipecac is not recommended because of the possibility of irritation or burns to the esophagus (Nadig 1994; Schonwald 2004).

In cases of dermal exposure, the skin should be thoroughly washed to prevent chromium absorption by the skin (Haddad et al. 1998; Leikin and Paloucek 2002; Schonwald 2004). As chromium(VI), but not chromium(III), is readily absorbed by the skin, ascorbic acid in the washing solution could reduce chromium(VI) to chromium(III), thus decreasing absorption. Application of the calcium disodium salt of ethylenediamine tetraacetic acid (EDTA), which acts as a chelating agent, has also been recommended after washing with water and application of ascorbic acid (Nadig 1994), especially in cases where the skin has been cut or abraded (Burrows 1983). Ascorbic acid was found to protect chromium-sensitive workers who handled chromates in the lithographing and printing industries from dermatitis. The ascorbic acid (10% solution) was kept near the work areas, and the workers soaked their hands and forearms as soon as possible after handling the chromate mixtures. In addition, ascorbic acid prevented ulcerations of the skin in rats treated with potassium dichromate dermally (Samitz 1970). An antichrome powder consisting of a mixture of 40% sodium metabisulfite, 20% ammonium chloride, 20% tartaric acid, and 20% sucrose as a 10% aqueous solution was effective in reducing the healing time of chrome sores on the skin of guinea pigs to which potassium dichromate had been applied (Samitz and Epstein 1962). Thorough irrigation with water has been recommended if the eyes have been exposed (Haddad et al. 1998; Schonwald 2004).

Both the cytotoxic effects of chromium(III) chloride, chromium(III) nitrate, sodium chromate(VI), sodium dichromate(VI), potassium dichromate(VI), and chromium(V) potassium sulfate dodecahydrate and the ability of ascorbic acid, glutathione 4-acetamido-4'-isothiocyanato-2,2-stibenedisolphonic acid (SITS) to prevent chromium toxicity in transformed human keratinocytes were examined (Little et al. 1996). This cell line was used because histopathological studies have shown that dichromate compounds have caused keratinocyte necrosis. Cells were exposed to the chromium salts for 24 hours, and the viability of the cultures was examined for their ability to take up neutral red dye and release lactate dehydrogenase into the media. None of the chromium(III) or chromium(V) salts seemed toxic to the cells at concentrations up to about 100 μM. The chromium(VI) salts showed toxicity at about 8 μM, and there was little cell survival at 100 μM. The dose-response curves were similar for all chromium(VI) salts tested. Similar experiments were conducted with normal human keratinocytes obtained from abdominoplasties or breast reductions from six donors and treated with sodium dichromate. The toxicity to normal cells overall seemed to be less than in the transformed line. Ascorbic acid at 500 μM completely inhibited the cell toxicity caused by chromium(VI), whereas glutathione and SITS were less effective. Ascorbate probably protected cells by reducing chromium(VI) and chelation of the reduced complex. Glutathione may have formed complexes with the chromium(VI), which eventually led to chromium(III), whereas SITS may have inhibited the cellular uptake of the chromate by altering the non-specific membrane anion carrier. The authors conclude that these available drugs provide protection against cytotoxicity to keratinocytes involved in dermatitis and may be useful to prevent toxic reactions to metals contacting the skin.

The effect of decreasing the concentration of water-soluble chromium in cement from about 10 to 2 ppm on the incidences of chromium-induced dermatitis was examined among construction workers in Finland (Roto et al. 1996). After 1987, when the decrease occurred, allergic dermatitis caused by chromium in the industry was reduced by 33% from previous levels, whereas irritant contact dermatitis remained unchanged.

3.11.2 Reducing Body Burden

Once chromium has been absorbed, it can be widely distributed throughout the body (see Section 3.4.2). Forced diuresis with careful monitoring of fluid and electrolyte status has been suggested, but not proven, to increase the elimination of chromates (Haddad et al. 1998). In a case report of a fatality after ingestion of potassium chromate, hemodialysis and charcoal hemoperfusion did not significantly remove chromium from whole blood and had little effect on the management of chromium toxicity (Iserson et al. 1983).

However, hemodialysis was effective in saving the life of an electroplater who accidentally swallowed plating fluid containing chromium trioxide (Fristedt et al. 1965). Because chromium may be sequestered in erythrocytes, exchange transfusion has been used as a way to decrease the body burden in serious acute poisoning (Kelly et al. 1982).

Both chromium(VI) and chromium(III) can be transported in the blood. Chromium(III) tends to bind to plasma proteins and is excreted in the urine. Chromium(VI) may be poorly reduced to chromium(III) in plasma, but this reduction can be enhanced by the intravenous administration of ascorbic acid (Korallus et al. 1984). However, reactions of chromium(VI) with sulfhydryl compounds or ascorbate may have mixed effects on toxicity, since such reactions yield reactive chromium intermediates, reactive oxygen species, and free cysteinyl and carbon radical species, which may be more damaging than chromium(VI) itself (Reynolds and Zhitkovich 2007; Shi et al. 1994; Stearns et al. 1994). Generally, treatments for reducing body burden of chromium are chelation therapies similar to those used to reduce body burdens of other metals, although the use of ascorbic acid is specific for chromium. Use of hemodialysis and N-acetylcysteine has been suggested to enhance elimination (Haddad et al. 1998; Leikin and Paloucek 2002; Schonwald 2004), however, this has not been proven. N-acetylcysteine, the glutathione precursor, was reported to be more effective than EDTA or dimercaptosuccinic acid in increasing the urinary excretion of chromium in rats (Banner et al. 1986; Nadig 1994); however, chelation with agents available in human clinical medicine, such as British Anti Lewisite (dimercaprol) and EDTA, has been shown to be generally ineffective in increasing the elimination of chromium (Ellis et al. 1982). However, calcium EDTA, administered intravenously, resulted in a rapid increase in the urinary excretion of chromium in metal workers (Sata et al. 1998). Other polyaminocarboxylic acid chelating agents may be effective in removing chromium from organs. In rats injected with potassium chromate, subsequent treatment with various polyaminocarboxylic acid chelating agents resulted in significant removal of chromium from the liver, kidney, heart, or brain, depending on the agent. Ethylenediamine N,N'-diacetic acid (EDDA) removed significant amounts of chromium from the liver and heart. Ethylenediamine N,N'-di (O-hydroxyphenyl acetic acid (EDDHA) removed significant amounts of chromium from the kidney, heart, and brain. N-(2-hydroxyethyl)ethylenediamine triacetic acid (HEDTA) removed significant amounts of chromium from the liver and kidney. Hexamethylene 1,6-diamino N,N,N',N'-tetraacetic acid (HDTA) removed significant amounts of chromium from the liver, kidney, and brain. Triethylene tetramine N,N,N',N',N'',N''-hexaacetic acid (TTHA) removed significant amounts of chromium from the liver. Ethyleneglycol-bis-(2-aminoethyl) tetraacetic acid (EGATA) did not remove significant amounts of chromium from any of the organs. The relative ability of the polyaminocarboxylic acids to remove chromium from organs may be related to the number of amino or carboxyl groups as complexing centers

or by the presence of hydroxyl groups (Behari and Tandon 1980). The use of these agents in humans has not been tested. Chromium(VI), but not chromium(III), can readily cross cell membranes. Chromium(VI) readily enters erythrocytes, where it is reduced to chromium(III) by glutathione, and chromium(III) is essentially trapped within erythrocytes, where it binds to proteins, primarily hemoglobin. This may explain the fact that chromium shows little toxicity at sites distant from administration sites (De Flora and Wetterhahn 1989). The chromium(III) trapped within the erythrocytes would be released upon natural destruction of the erythrocyte and excreted in the urine.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The reduction of chromium(VI) to chromium(III) inside of cells may be an important mechanism for the toxicity of chromium, whereas reduction of chromium(VI) outside of cells may be a major mechanism of protection. After entering target cells, chromium(VI) itself and/or the metabolically reduced valence states exert toxic effects, as discussed in detail below (De Flora and Wetterhahn 1989). Administration of a reducing agent (such as ascorbate) early enough after exposure to reduce chromium(VI) to chromium(III) in extracellular fluids before chromium(VI) penetrates cells may reduce toxicity; however, increased intracellular ascorbate may enhance toxicity. For example, in animal studies, ascorbic acid has been shown to protect against lethality of dermal potassium dichromate (Samitz 1970) and prevent nephrotoxicity of subcutaneously administered potassium chromate (Powers et al. 1986). However, increased intracellular ascorbate concentrations has been shown to enhance chromium(VI) toxicity in cultured human fibroblasts (Reynolds and Zhitkovich 2007). Therefore, agents that enhance reduction of chromium(VI) to chromium(III) may have mixed effects on toxicity. The effect of ascorbate or other reducing agents on chromium toxicity in humans has not been established.

Once chromium enters the cell, ligand displacement and/or redox reactions of chromium(VI) with enzymes, proteins, and other molecules leads to reduction to chromium(V), chromium(IV), and chromium(III), with the generation of active oxygen species and radicals. The resulting toxicity depends on the nature of the cellular component that reacts with chromium(VI) and on the nature of the reactive species formed from the reaction. Chromium(VI) can be reduced metabolically by a number of cellular components under physiological conditions. Reduction by glutathione or cysteine can lead to generation of all valence states (particularly chromium(V)) and radicals. For example, *in vitro* reaction of chromium(VI) with glutathione led to the formation of glutathione thiyl radicals and chromium(V) complexes (Aiyar et al. 1991). Chromium(V)-glutathione complexes have been shown to form DNA adducts. Reduction by ascorbate leads to chromium(III), but chromium(V) has been generated by the

reaction of chromium(VI) with riboflavin (vitamin B_2) and ribose derivatives (De Flora and Wetterhahn 1989). Reaction of chromium(VI) with hydrogen peroxide has led to the formation of chromium(V) complexes and hydroxyl radicals (Aiyar et al. 1991). Other important intracellular reduction reactions of chromium(VI) involve enzyme-catalyzed and NADPH-dependent mechanisms. Microsomal reduction of chromium(VI) by cytochrome P450 to chromium(III) may involve the transient formation of chromium(V) (De Flora and Wetterhahn 1989). Chromium(III), the final stable product of chromium(VI) reduction, can form chromium-DNA adducts and mediate crosslinking of DNA strands and DNA protein (De Flora and Wetterhahn 1989; Manning et al. 1992; Xu et al. 1996). Thus, the metabolic reduction of chromium(VI) may represent bioactivation and/or detoxification. If a bioactivation process, intracellular reduction of chromium(VI) would lead to the ultimate toxic species. Conversely, if chromium(VI) is the toxic agent, then effects would be elicited only if the amount of chromium(VI) entering target cells saturates the reducing mechanisms.

Differences in the intracellular metabolic pathways that result in the reduction of chromium(VI) will affect the nature of the reactive intermediates. For example, chelating ligands, such as glutathione and sugars, stabilize chromium(V) as an oxidation state, increasing its lifetime in the cell and ability to reach DNA in the nucleus. Cytochrome P450-dependent reduction of chromium(VI) to chromium(V) and chromium(IV), with generation of reactive radicals, which takes place in the endoplastic reticulum, could occur in close enough proximity to the nuclear membrane and nonenzymatic reduction within the nucleus could occur in close enough proximity to chromatin for the transient intermediates to exert their effects, such as DNA strand breaks and radical-DNA adducts. As noted above, chromium(III) can form chromium-DNA adducts and mediate crosslinking of DNA strands and DNA protein (De Flora and Wetterhahn 1989).

The role of glutathione in chromium-induced renal toxicity was investigated by Hojo and Satomi (1991). Male ddY mice (6 animals per dose group) were administered potassium dichromate(VI) (0.6 mmol chromium/kg), potassium tetraperoxochromate(V) (1.0 mmol/kg), green chromium(V)-glutathione complex (1.0 mmol/kg), and chromium nitrate (III) (0.6 mmol/kg); animals were sacrificed 24 hours after chromium injection and changes in kidney weight and function were assessed. Chromium(VI) resulted in a 10.7%±2.7 decrease in body weight, a 2-fold increase in serum urea nitrogen, a decrease in kidney nonprotein sulfhydryl contents (3.3±0.1 versus control values of 3.7±0.1) and a decrease of kidney-glutathione reductase activity from a control value of 17.4±1.5 to 14.1±1.3 U/g. Potassium tetraperoxochromate(V) treatment resulted in 50% of the animals dying. Body weights and kidney-glutathione reductase activity were much lower than for animals treated with chromium(VI), and serum

urea levels were 102.9±17.7 mg/dL, which is about twice that observed in animals treated with chromium(VI). The chromium(V) glutathione complex was much less toxic and showed values that were similar or close to control values. Pretreatment with 10 mmol/kg glutathione methyl ester in the chromium(VI)-treated animals appeared to reduce the body weight loss and caused the serum urea levels to be normal. Butathione sulfoximine (an inhibitor of glutathione synthesis) greatly enhanced the levels of serum urea, loss of glutathione reductase activity and decrease in kidney nonprotein sulfhydryl groups. Butathione sulfoximine pretreatment resulted in one of the six animals dying. Animals treated with chromium(III) experienced weight loss, but other parameters were not markedly changed from control values. Pretreatment with butathione sulfoximine in animals treated with chromium(III) only caused a decrease in kidney nonprotein sulfhydryl groups. The authors indicated that with excess levels of glutathione, chromium(VI) is more readily reduced to chromium(III), whereas at lower levels of glutathione the reduction process is slower, resulting in slower reduction of the more toxic intermediate chromium(V). Also, at higher concentrations of glutathione, chromium(V)-glutathione complexes may form which may prevent chromium(V) from reacting at target sites that elicit toxic responses.

As discussed above, reactive intermediates formed during intracellular reduction of chromium(VI) to chromium(III) may interact with hydrogen peroxide, generating hydroxyl radicals, which can induce cell damage. Several animal and *in vitro* studies have assessed the effects of anti-oxidant agents on chromium-induced oxidative cell injury. Administration of folic acid, a free radical scavenger, to rabbits reduced potassium dichromate-induced increases in the concentration of free radical in liver, testes, brain, kidney, and lung and in serum liver enzyme activities of AST and ALT (El-Demerdash et al. 2006). Vitamin E, an antioxidant, has been shown to reduce potassium dichromate-induced renal toxicity and hepatotoxicity in rats (Appenroth et al. 2001; Arreola-Mendoza et al. 2006; Rao et al. 2006). Vitamin B_6, which may have anti-oxidant potential due to its role as a co-factor in the synthesis of cysteine, reduced oxidative stress in the liver of rats exposed to potassium dichromate (Anand 2005). *In vitro* studies indicated that vitamin E protected against, while vitamin B_2 enhanced, the cytotoxicity and DNA single-strand breaks induced by sodium chromate in Chinese hamster cells. Formation of DNA-protein crosslinks by chromium(VI) in cell culture was prevented by addition of ascorbic acid (Capellmann et al. 1995), and ascorbic acid protected cells against chromosomal breakage and apoptosis. Vitamin E also protected cells against chromosomal breaks (Blankenship et al. 1997) and decreased chromium(III)-induced oxidative damage to calf thymus DNA *in vitro*, as indicated by decreased formation of 8-hydroxydeoxyguanosine (Qi et al. 2000). Vitamin E may exert its protective effect by scavenging radicals and/or chromium(V) during the reduction of chromium(VI) (Sugiyama 1991). Selenium (as sodium selenate), an essential trace element, has been shown to reduce the genotoxicity of chromium

dichromate in human lymphocytes *in vitro* as assessed by the Comet assay, although sodium selenite and selenous acid enhanced chromium-induced DNA damage; sodium selenate also decreased chromium-induced genotoxicity in *S. typhmuirium* (strain TA102), as assessed by the Ames assay (Cemeli et al. 2003). Other vitamins or essential elements might also be effective in mitigating the effects of chromium by modulating the metabolic processes. The use of vitamins and essential elements for reducing the toxicity of chromium has not been studied in humans.

Thyroxine was found to ameliorate acute renal failure induced in rats by potassium dichromate, possibly by stimulating gluconeogenesis and Na-K ATPase activity in the renal cortex, influencing protein synthesis, and promoting glucose and amino acid uptake by epithelial cells. These events would be expected to aid in the repair and regeneration of the damaged tubular epithelial cells (Siegel et al. 1984). The use of thyroxine has not been tested in humans.

Todralazine, an antihypertensive drug, was found to markedly reduce the mutagenic activity of potassium dichromate (VI) in the bacterial tester strain TA100 and in the *B. subtilis* rec assay (Gasiorowski et al. 1997). Spectroanalysis indicated that chromium(VI) was reduced to chromium(III) by todralazine and that todralazine formed a complex with the chromium(III) ions. The reduction and complexing of chromium may have prevented chromium from crossing the membrane and may have prevented harmful interactions with DNA. Another study by this group found that complexing copper(II) chromate(VI) to organic ligands (e.g., 2-(2'-pyridyl)imidazole, 2,2'-bipyridyl, 1,10-phenanthroline) resulted in a decrease in the mutagenicity of chromium(VI) as assessed by the Ames and *B. subtilis* rec assays (Gasiorowski et al. 1998).

3.12 ADEQUACY OF THE DATABASE

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

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would

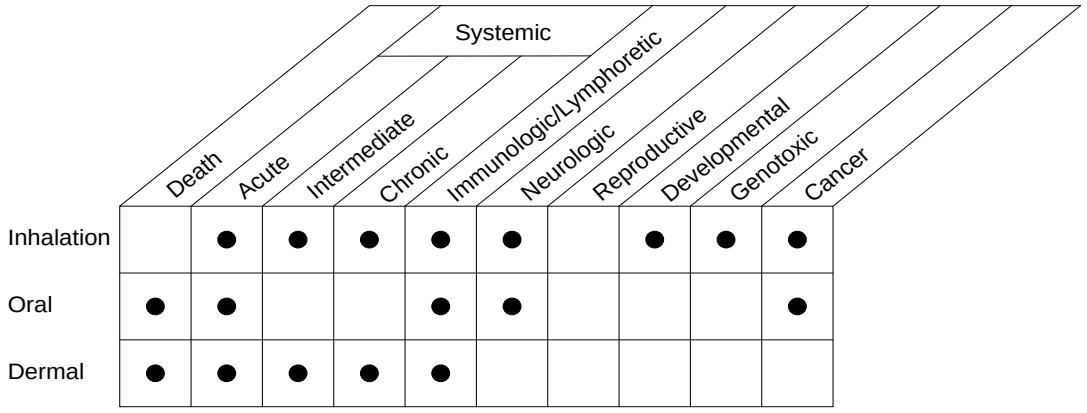
reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Chromium

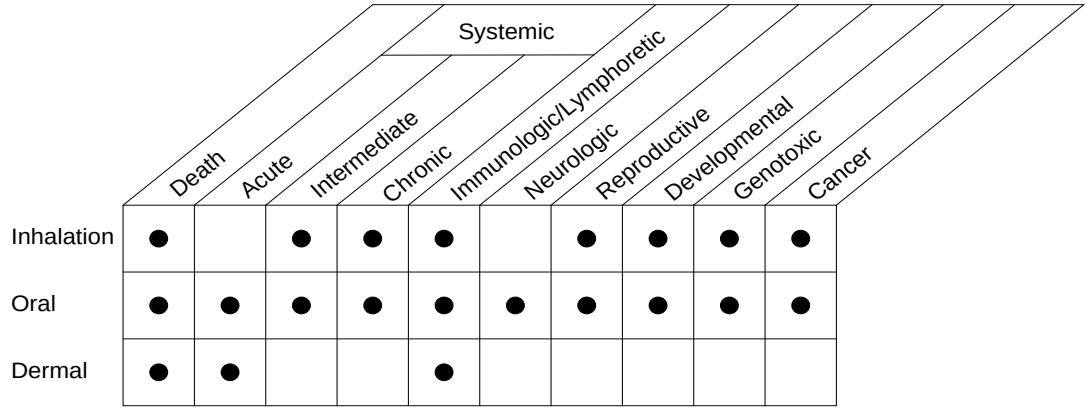
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chromium are summarized in Figures 3-8 and 3-9. The purpose of these figures is to illustrate the existing information concerning the health effects of chromium. Each dot in the figures indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

A major source of oral exposure of humans to chromium is via the diet including chromium-rich diet supplements. Chromium(III) at low levels is essential to nutrition, and studies of chromium deficiency have been conducted. Information regarding health effects of exposure to chromium(VI) or chromium(III) in humans comes mainly from case reports of acute accidental or intentional ingestion, acute accidental dermal exposure, and from occupational case reports and epidemiology studies, which primarily involve inhalation and dermal exposure. In occupational studies, it is often difficult to separate exposure to chromium(VI) from chromium(III). Case reports have shown that ingestion and dermal contact with chromium(VI) can cause death. These reports have also described the serious systemic and neurological sequelae of exposure leading to death. Occupational exposures to chromium(VI) and/or chromium(III) are associated with respiratory and nasal, cardiovascular, gastrointestinal, hematological, hepatic, renal, and dermal effects. Immunological effects in humans exposed by inhalation and dermal contact consist of sensitization resulting in asthma and contact dermatitis, which can be exacerbated by oral exposure. Limited information was available regarding reproductive effects of occupational exposure to chromium(VI). Limited information was found on neurological behavioral effects. Information is also available regarding genotoxic effects in workers exposed to chromium(VI) and cancer in workers exposed to chromium(VI) and/or chromium(III).

Figure 3-8. Existing Information on Health Effects of Chromium(VI)



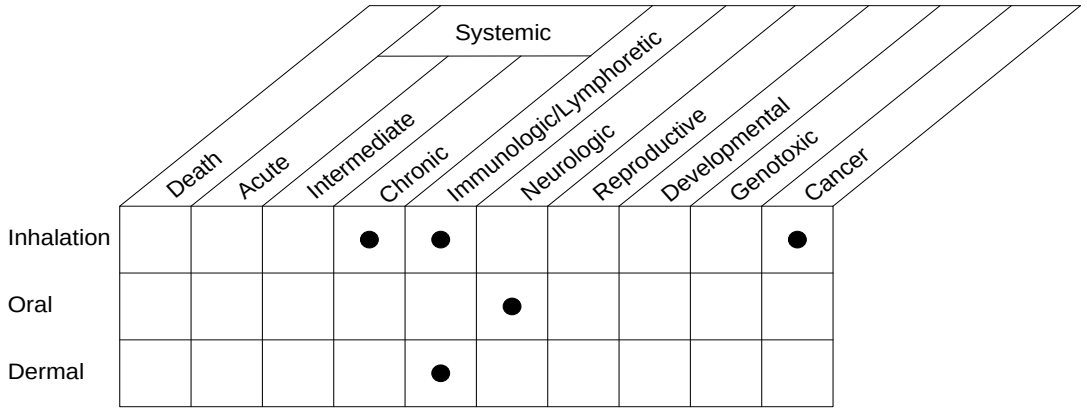
Human



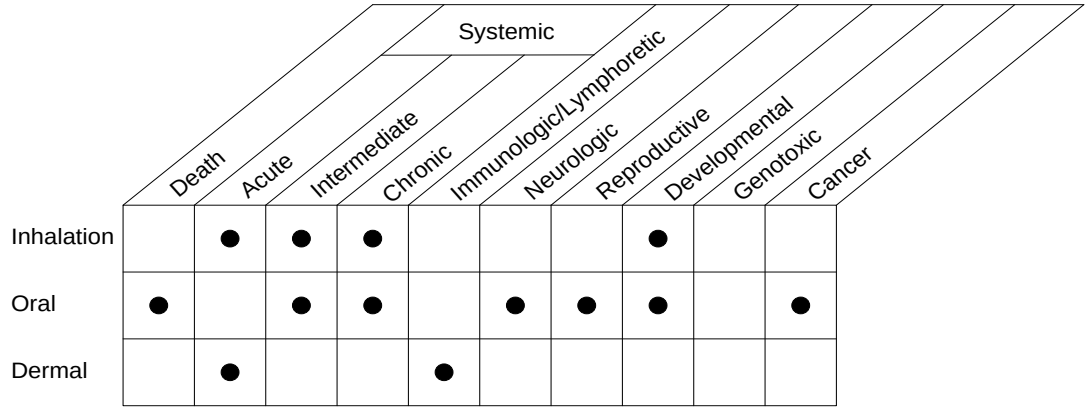
Animal

● Existing Studies

Figure 3-9. Existing Information on Health Effects of Chromium(III)



Human



Animal

● Existing Studies

Information regarding the levels of exposure to chromium(VI) compounds that cause death in animals is available for the inhalation, oral, and dermal routes. Information regarding respiratory effects of acute inhalation exposure of animals to chromium(VI) was available. Acute oral studies have evaluated effects of chromium(VI) on hematology and clinical chemistry. Acute dermal exposure of animals to chromium(VI) can cause irritation, edema, necrosis, and chrome sores. Information on systemic effects of chromium(VI) in animals is available for intermediate- and chronic-duration exposure by the inhalation route. Information regarding effects of oral exposure is available for intermediate and chronic durations. The immunological effects of chromium(VI) in animals have been studied after inhalation and dermal exposure. An inhalation study reported no developmental or reproductive effects of chromium(VI). The reproductive and developmental effects of oral chromium(VI) have been evaluated following oral exposure, showing adverse effects, particularly to the male reproductive system. Information regarding the genotoxicity and carcinogenicity of chromium(VI) is available for both the inhalation and oral routes.

Information regarding levels of chromium(III) compounds that result in death is available only for the oral route. Systemic effects of acute-duration exposure to chromium(III) are limited to the respiratory system- and intermediate-duration inhalation exposure to chromium(III) are limited to the respiratory system. Information on systemic effects of chronic inhalation exposure to chromium(III) is limited to a study that used a mixture of chromium(VI) and chromium(III). Studies of intermediate- and chronic duration oral exposure to chromium(III) failed to find any systemic, neurological, developmental, reproductive, or carcinogenic effects. The immunological and genotoxic effects of chromium(III) in animals have not been tested by the oral route. Information regarding effects of dermal exposure of animals to chromium(III) is limited to a study of skin ulceration after acute exposure and dermal sensitization tests. One report of chronic renal failure after ingestion of over-the-counter chromium picolinate at 0.6 mg/day was found in literature (Wasser et al. 1997).

In addition to the information on chromium(VI) and chromium(III), limited information is available regarding health effects of chromium(0) and chromium(IV). Briefly, the available information on chromium(0) consists of studies that examined workers at an alloy steel plant (Triebig et al. 1987) and boilermakers (Verschoor et al. 1988) for possible renal effects. Information on chromium(IV) consists of a 2-year inhalation study of chromium dioxide in rats that found no effects upon hematological, clinical chemistry, and urinalysis parameters and no histopathological effects on respiratory, cardiovascular, gastrointestinal, hepatic, renal, dermal/ocular, neurological, and reproductive organs (Lee et al. 1989).

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Acute inhalation exposure of humans to chromium(VI) as occurs in occupational settings can result in respiratory irritation (dyspnea, cough, wheezing, sneezing, rhinorrhea, choking sensation), dizziness, and headache at high concentrations, and can trigger asthmatic attacks in sensitized individuals (Lieberman 1941; Meyers 1950; Novey et al. 1983; Olaguibel and Basomba 1989). High airborne levels of chromium(VI) can also cause gastrointestinal irritation (Lucas and Kramkowski 1975; Mancuso 1951; Meyers 1950). Information on toxic effects in humans after oral exposure to chromium(VI) is limited to case reports of humans who ingested lethal or near lethal doses. Serious respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects have been described as sequelae leading to death (Clochesy 1984; Iserson et al. 1983; Kaufman et al. 1970; Saryan and Reedy 1988). Acute dermal exposure can cause skin burns and can also have similar sequelae that lead to death (Brieger 1920; Major 1922). No information regarding systemic effects of acute inhalation exposure of animals to chromium(VI) was located. Information regarding effects of acute oral exposure of animals to chromium(VI) include a report of gastrointestinal hemorrhage in rats given a lethal dose of potassium dichromate (Samitz 1970), evaluations of hematology and clinical chemistry parameters in rats and mice exposed for 4–5 days (NTP 2007, 2008a) and increased resorptions in mice given potassium dichromate during gestation (Junaid et al. 1996b). Information regarding effects of acute dermal exposure of animals to chromium(VI) is limited to studies of dermal irritation and sensitization (Gad et al. 1986; Merkur'eva et al. 1982; Samitz 1970; Samitz and Epstein 1962). The information in humans indicates that many organs can be targets of acute exposure to chromium(VI) if exposure levels are high enough. Studies in animals show that hematological effects occur following acute oral exposure and may be the earliest indication of more severe adverse effects observed following longer duration exposures. No information was located regarding systemic effects in humans after acute exposure to chromium(III) compounds by any route. Acute inhalation studies of chromium trichloride in hamsters (Henderson et al. 1979) and chromic oxide and basic chromium sulfate in rats (Derelanko et al. 1999) indicated that the respiratory system is also a target of chromium(III) exposure. Acute dermal studies show that chromium(III) can be a sensitizer, and that dermal challenge of sensitized individuals with chromium(III) compounds can elicit a response (Hansen et al. 2003; Samitz and Epstein 1962). LD_{50} values for chromium(VI) and chromium(III) compounds indicate that chromium(III) is less toxic than chromium(VI) (Shubochkin and Pokhodzie 1980; Smyth et al. 1969; Vernot et al. 1977).

Additional studies involving acute exposure to both chromium(VI) and chromium(III) compounds by all routes would be helpful, especially if they evaluated comprehensive toxicological end points and

exposure-response relationships. Studies defining the possible synergistic effects of chromium with other nephrotoxins, such as mercury and cadmium, which may be stored together at toxic waste sites, would also be useful. There are populations surrounding hazardous waste sites that might be exposed to the substance for short periods; therefore, this information is important.

Intermediate-Duration Exposure. There are no studies regarding systemic effects in humans after oral exposure of intermediate duration to either chromium(VI) or chromium(III). Intermediate-duration exposure to primarily chromium(VI) in occupational studies caused nasal and respiratory effects (Bovet et al. 1977; Davies et al. 1991; Gomes 1972; Kleinfeld and Rosso 1965; Lee and Goh 1988; Sorahan et al. 1987; Taylor 1966). Intermediate-duration exposure in occupational settings involving dermal exposure also can cause chrome ulcers or holes in the skin (Gomes 1972; Lee and Goh 1988; Lieberman 1941; PHS 1953; Smith 1931). An MRL of $5x10^{-6}$ mg chromium(VI)/m^3 has been determined for upper respiratory effects in humans after intermediate-duration inhalation exposure to chromium(VI) as chromium(VI) trioxide mist and other hexavalent chromium mists and dissolved aerosols, based on the study by Lindberg and Hedenstierna (1983).

The respiratory tract and the immune system are targets in animals exposed to chromium(VI) and chromium(III) via inhalation for intermediate durations (Adachi 1987; Adachi et al. 1986; Glaser et al. 1985, 1990; Johansson et al. 1986a, 1986b), with LOAEL values identified for respiratory and immune effects after inhalation (Glaser et al. 1985, 1990). An MRL of 0.0003 mg chromium(VI)/m^3 has been determined for lower respiratory effects in humans after intermediate-duration inhalation exposure to chromium(VI) as particulate hexavalent compounds based on the study in rats by Glaser et al. (1990). An intermediate-duration study on chromium(III) compounds in rats identified respiratory system as the target for inhaled insoluble chromic oxide and soluble basic chromium sulfate (Derelanko et al. 1999). Based on differences in respiratory effects of these two compounds, distinct intermediate-duration MRLs were derived for insoluble and soluble trivalent chromium compounds. The minimal LOAEL of 3 mg chromium(III)/m^3 for septal cell hyperplasia and chronic interstitial inflammation of the lung in male rats exposed to chromic oxide was used to derive the intermediate-duration inhalation MRL of 0.005 mg chromium(III)/m^3 for insoluble trivalent chromium compounds. The LOAEL of 3 mg chromium(III)/m^3 for lesions of the larynx (granulomatous inflammation) and nose (inflammation) in female rats exposed to basic chromium sulfate was used to derive the intermediate-duration inhalation MRL of 0.0001 mg chromium(III)/m^3 for soluble trivalent chromium compounds.

The gastrointestinal and hematological systems were identified as the primary targets of intermediate-duration oral exposure of rats and mice exposed to chromium(VI) in drinking water (NTP 2007, 2008a). An intermediate-duration oral MRL of 0.005 mg chromium(VI)/kg/day has been determined for hematological effects (e.g., microcytic, hypochromic anemia) in rats after intermediate-duration oral exposure to chromium(VI) as sodium dichromate dihydrate in drinking water in a study by NTP (2008a). In addition, developmental and reproductive studies identify chromium(VI) as a reproductive and developmental toxicant in monkey, rats, and mice after oral exposure (Al-Hamood et al. 1998; Aruldhas et al. 2004, 2005, 2006; Bataineh et al. 1997; Chowdhury and Mitra 1995; Junaid et al. 1996b; Kanojia et al. 1996, 1998; Subramanian et al. 2006; Trivedi et al. 1989; Yousef et al. 2006; Zahid et al. 1990). Oral studies of intermediate-duration in rats and mice reported no effects of chromium(III) in any system (Ivankovic and Preussmann 1975; NTP 2008b; Shara et al. 2005). Adverse reproductive effects were observed following oral exposure to chromium(III), although NOAEL values were not established. No dermal studies of intermediate duration in animals were located. The toxicity of intermediate-duration exposure to chromium compounds is relatively well characterized for the oral and inhalation routes. Dermal studies would be useful to determine possible target organs other than the skin. There are populations surrounding hazardous waste sites that might be exposed to the substance for similar durations.

Chronic-Duration Exposure and Cancer. The respiratory system (Bovet et al. 1977; Cohen et al. 1974; Davies et al. 1991; Gibb et al. 2000a; Keskinen et al. 1980; Kleinfeld and Rosso 1965; Kuo et al. 1997a; Letterer 1939; Lindberg and Hedenstierna 1983; Lucas and Kramkowski 1975; Mancuso 1951; Meyers 1950; Novey et al. 1983; Olaguibel and Basomba 1989; Sassi 1956; Sluis-Cremer and du Toit 1968; Sorahan et al. 1987; Taylor 1966) and the skin (Gomes 1972; Hanslian et al. 1967; Lee and Goh 1988; Lieberman 1941; PHS 1953; Royle 1975b) are the primary target organs for occupational exposure to chromium and its compounds. An MRL of $5x10^{-6}$ mg chromium(VI)/m^3 has been determined for upper respiratory effects in humans after chronic-duration inhalation exposure to chromium(VI) as chromium(VI) trioxide mist and other hexavalent chromium mists and dissolved aerosols, based on the study by Lindberg and Hedenstierna (1983). There are more data regarding the effects of chronic inhalation exposure in humans and animals than there are regarding the effects of oral exposure. Studies of populations residing in areas contaminated with chromium(VI) in China have found such effects as oral ulcer, diarrhea, abdominal pain, indigestion, vomiting, constipation, nose and eye irritation, headache, fatigue, dizziness, and leukocytosis (Zhang and Li 1987). Chronic inhalation studies with rats, mice, guinea pigs, and rabbits also identify the respiratory system as the main target of chromium(VI) and chromium(III) exposure (Glaser et al. 1986, 1988; Nettesheim and Szakal 1972; Steffee and Baetjer

1965). Chronic oral exposure studies in rats and mice exposed to chromium(VI) in drinking water identify the hematological and gastrointestinal systems as the primary targets of chronic oral exposure (NTP 2008a), with gastrointestinal effects more sensitive than hematological effects. A chronic-duration oral MRL of 0.001 mg chromium(VI)/kg/day based on gastrointestinal effects (diffuse epithelial hyperplasia of the duodenum) was derived for hexavalent chromium compounds. Chronic oral exposure to chromium(III) compounds did not result in any target organ toxicity in animals (Ivankovic and Preussmann 1975; MacKenzie et al. 1958; NTP 2008b; Schroeder et al. 1965; Shara et al. 2007); thus, no chronic-duration MRL was derived for chromium(III) compounds since target organs have not been identified and no NOAEL for reproductive effects of oral exposures has been adequately characterized. As noted above, the skin is a sensitive target of toxicity in workers exposed to airborne chromium (the effects resulted from direct dermal contact with chromium). No chronic dermal studies in animals were located. Because water and soil sources can be contaminated near hazardous waste sites, more information regarding chronic oral or dermal exposure would be useful.

Cancer. Occupational and environmental epidemiological studies indicate a correlation between long-term exposure to chromium(VI) compounds and lung cancer (Alderson et al. 1981; Baetjer 1950b; Bidstrup 1951; Bidstrup and Case 1956; Braver et al. 1985; Cole and Rodu 2005; Crump et al. 2003; Dalager et al. 1980; Davies 1979, 1984; Davies et al. 1991; EEH 1976, 1983; Enterline 1974; Franchini et al. 1983; Frentzel-Beyme 1983; Gibb et al. 2000b; Goldbohm et al. 2006; Haguenoer et al. 1981; Hayes et al. 1979, 1989; Korallus et al. 1982; Langård and Norseth 1975; Langård and Vigander 1983; Langård et al. 1980; Machle and Gregorius 1948; Mancuso 1975, 1997a; Mancuso and Hueper 1951; Ohsaki et al. 1978; Park and Stayner 2006; Park et al. 2004; Pastides et al. 1994; PHS 1953; Rosenman and Stanbury 1996; Sassi 1956; Satoh et al. 1981; Sheffet et al. 1982; Silverstein et al. 1981; Sjogren et al. 1987; Sorahan et al. 1987; Taylor 1966; Zhang and Li 1987). Occupational studies generally consider inhalation exposures, while environmental studies involve exposure by inhalation, ingestion, and dermal contact. Additional studies on populations exposed to chromium in drinking water would be useful to determine if a causal relationship with cancer exists. A unit risk for cancer from inhalation exposure to chromium(VI) compounds has been derived (IRIS 2008) from an occupational study (Mancuso 1975). Chronic inhalation of chromium(VI) compounds was carcinogenic in rats (Glaser et al. 1986) and mice (Nettesheim et al. 1971), and the 2-year carcinogenicity study on oral chromium(VI) provided clear evidence of oral cancers in rats and gastrointestinal cancers in mice (NTP 2008a). Cancer studies by parenteral route support the conclusions that chromium(VI) is carcinogenic (Furst et al. 1976; Hueper 1955, 1958; Hueper and Payne 1959, 1962; Laskin et al. 1970; Levy et al. 1986; Roe and Carter 1969; Steinhoff et al. 1986). For chromium(III) compounds, evidence for carcinogenesis (preputial adenomas in

male rats) in the NTP (2008b) 2-year bioassay was equivocal. The available human and animal data are sufficient for determining that chromium(VI) is carcinogenic following inhalation and oral exposure. However, additional animal studies are needed to adequately assess the carcinogenic potential of chromium(III) following inhalation and oral exposure.

Genotoxicity. Several studies evaluating chromosomal aberrations, sister chromatid exchange, micronuclei, DNA strand breaks and DNA-protein crosslinks in workers exposed to chromium(VI) have been conducted, some reporting positive results (Benova et al. 2002; Deng et al. 1988; Gambelunghe et al. 2003; Koshi et al. 1984; Lai et al. 1998; Medeiros et al. 2003a; Sarto et al. 1982; Stella et al. 1982; Vaglenov et al. 1999; Werfel et al. 1998; Wu et al. 2001) and some reporting negative results (Benova et al. 2002; Gao et al. 1994; Hamamy et al. 1987; Husgafvel-Pursiainen et al. 1982; Littorin et al. 1983; Medeiros et al. 2003a; Nagaya 1986; Nagaya et al. 1991). However, most of these studies are limited by factors such as lack of exposure data, co-exposure to other potentially genotoxic agents, and too few workers for meaningful statistical analysis. Mostly positive results have been found in rodents and *D. melanogaster* exposed to chromium(VI) compounds *in vivo* (De Flora et al. 2006; Gava et al. 1989a; Itoh and Shimada 1993; Kaya et al. 2002; Kirpnick-Sobol et al. 2006; Mirsalis et al. 1996; NTP 2007; Olvera et al. 1993; Paschin et al. 1982; Rasmuson 1985; Rodriguez-Arnaiz and Martinez 1986; Sarkar et al. 1993; Shindo et al. 1989; Tsapakos et al. 1983b; Ueno et al. 2001; Wang et al. 2006; Wild 1978; Zimmering et al. 1985). Numerous *in vitro* genotoxicity studies have been conducted in bacteria (Bennicelli et al. 1983; De Flora 1978, 1981; Haworth et al. 1983; Kanematsu et al. 1980; Kortenkamp et al. 1996b; Llagostera et al. 1986; Nakamuro et al. 1978; Nishioka 1975; NTP 2007; Olivier and Marzin 1987; Tagliari et al. 2004; Venier et al. 1982; Venitt and Levy 1974; Watanabe et al. 1998a; Yamamoto et al. 2002), yeast (Bonatti et al. 1976; Fukunaga et al. 1982; Kirpnick-Sobol et al. 2006; Singh 1983), cultured animal cell systems (Briggs and Briggs 1988; DiPaolo and Casto 1979; Douglas et al. 1980; Elias et al. 1989b; Fornace et al. 1981; Kowalski et al. 1996; Levis and Majone 1979; MacRae et al. 1979; Montaldi et al. 1987; Newbold et al. 1979; Ohno et al. 1982; Raffetto et al. 1977; Seoane and Dulout 1999; Sugiyama et al. 1986a; Tsuda and Kato 1977; Ueno et al. 1995a; Umeda and Nishimura 1979; Venier et al. 1982; Wise et al. 1993; Yang et al. 1992), and human cell systems (Blasiak and Kowalik 2000; Depault et al. 2006; Douglas et al. 1980; Fornace et al. 1981; Gomez-Arroyo et al. 1981; Ha et al. 2004, 2004; Holmes et al. 2006; MacRae et al. 1979; Montaldi et al. 1987; Nakamuro et al. 1978; Sarto et al. 1980; Stella et al. 1982; Sugiyama et al. 1986a; Trzeciak et al. 2000; Whiting et al. 1979; Wise et al. 2002, 2004, 2006a, 2006b), mostly with positive results. The vast majority of studies, therefore, clearly indicated that chromium(VI) compounds are genotoxic.

Genotoxicity data are also available for chromium(III) compounds. A study in tannery workers, who were exposed mainly to chromium(III), reported negative results for chromosomal aberrations and sister chromatid exchange (Hamamy et al. 1987), while positive results for micronuclei and DNA-protein crosslinks were reported in another study on tannery workers (Medeiros et al. 2003a). Chromium trichloride, chromium picolinate, and niacin-bound chromium(III) also did not cause DNA damage, or increased frequencies of micronuclei in rats exposed *in vivo* (Cupo and Wetterhahn 1985; De Flora et al. 2006; NTP 2008b; Shara et al. 2005). Transplacental exposure to chromium(III) chloride salt resulted in DNA deletions (Kirpnick-Sobol et al. 2006). Mostly negative results have been found in *in vitro* genotoxicity studies of chromium(III) compounds in bacteria (Bennicelli et al. 1983; De Flora 1981; Kanematsu et al. 1980; Llagostera et al. 1986; Matsui 1980; Nishioka 1975; NTP 2008b; Olivier and Marzin 1987; Petrilli and De Flora 1978b; Shara et al. 2005; Venier et al. 1982, 1989; Yamamoto et al. 2002), and mammalian cell systems (Fornace et al. 1981; Itoh and Shimada 1996; Le Curieux et al. 1992; Levis and Majone 1979; MacRae et al. 1979; Newbold et al. 1979; Ohno et al. 1982; Raffetto et al. 1977; Sarkar et al. 1993; Sarto et al. 1980; Shara et al. 2005; Stella et al. 1982; Tsuda and Kato 1977; Ueno et al. 1995a; Umeda and Nishimura 1979; Whiting et al. 1979; Wise et al. 1993; Yang et al. 1992). Chromium(III) did not increase the number of micronuclei in polychromatic erythrocytes in mice (Itoh and Shimada 1996). Several studies have found weakly positive or positive results in Chinese hamster ovary cells (Coryell and Stearns 2006; Levis and Majone 1979; Stearns et al. 2002), mouse fetal cells (Raffetto et al. 1977), mouse lymphoma cells (Whittaker et al. 2005), and human cell lines (Blasiak and Kowalik 2000; Nakamuro et al. 1978; Stella et al. 1982).

Chromium(III) compounds are less genotoxic than chromium(VI) compounds in intact cell systems because of the relative inability of chromium(III) to cross cell membranes; however, chromium(III) is more genotoxic than chromium(VI) when tested *in vitro* in subcellular targets (Kowalski et al. 1996; Snow 1991; Snow and Xu 1989). The reduction of chromium(VI) to chromium(III) as the ultimate genotoxicant within cells may account for the genotoxicity of chromium(VI) (Beyersmann and Koster 1987). However, in intact cells, chromium(III) appears less genotoxic than chromium(VI) due to decreased cellular permeability to chromium(III).

Additional studies in workers with known levels of chromium exposure that control for confounding factors would be useful for defining levels at which chromosomal aberrations occur in humans exposed to chromium(VI) in the workplace. Also, better dose-response relationships would be useful for the various genotoxic and regulatory effects observed with chromium to better determine which end points are the most sensitive and dominant at exposures near environmental levels.

Reproductive Toxicity. No reliable information was located regarding reproductive effects in humans after inhalation, oral, or dermal exposure to chromium or its compounds. Studies in women exposed occupationally also show that chromium can be transferred to fetuses through the placenta (Shmitova 1980). Inhalation studies would be useful for determining the reproductive toxicity of inhaled chromium and compounds and for establishing exposure-response relationships. Adverse effects on the male reproductive system (included decreased spermatogenesis and histopathological alterations to the epididymis) were observed in monkeys exposed to chromium(VI) in drinking water for 180 days (Aruldhas et al. 2004, 2005, 2006; Subramanian et al. 2006). Effects on spermatogenesis were reported in male rats given chromium(VI) by gavage for 90 days (Chowdhury and Mitra 1995) and in rabbits exposed to chromium(VI) in drinking water for 10 weeks (Yousef et al. 2006). In male mice, oral exposure of intermediate duration to chromium(VI) or chromium(III) was reported to result in decreased spermatogenesis and cellular degeneration of the outer layer of seminiferous tubules (Zahid et al. 1990); alterations in testicular, seminal vesicle, and preputial gland weights and decreased fertility were observed in mice following intermediate-duration exposure to chromium(VI) or chromium(III) (Elbetieha and Al-Hamood 1997). However, results of the study by Elbetieha and Al-Hamood 1997 should be interpreted with caution due to concern regarding experimental methods (see discussion in Section 2.3, Minimal Risk Levels). But other studies found no reproductive effects in male or female mice (NTP 1996a, 1996b, 1997, 2007, 2008a) exposed to chromium(VI) or chromium(III) (NTP 2008b; Shara et al. 2005, 2007). Alterations in sexual behavior and aggressive behavior toward other males were observed in male rats exposed to chromium(VI) or chromium(III) (Bataineh et al. 1997). Female mice or rats exposed orally to chromium(VI) compounds prior to mating (Junaid et al. 1996a; Kanojia et al. 1996, 1998) or female mice exposed during gestation (Junaid et al. 1996b; Trivedi et al. 1989) had increased fetal resorptions and decreased litter size. Alterations in ovarian and uterine weights and impaired fertility were observed in female mice that were exposed to chromium(III) or chromium(VI) and then were mated with unexposed mice (Elbetieha and Al-Hamood 1997) ; however, these results should be interpreted with caution due to concern regarding experimental methods (see discussion in Section 2.3, Minimal Risk Levels). Reductions in numbers of follicles and ova/mouse were seen following oral chromium(III) exposure (Murthy et al. 1996). Impaired development of the reproductive system was observed in the female offspring of mice exposed to potassium dichromate(VI) or chromium(III) chloride (Al-Hamood et al. 1998). A decrease in the number of pregnancies was observed in female rats administered 33.6 mg chromium(III)/kg/day as chromium chloride (by gavage) on gestational days 1–3; the same treatment on gestational days 4–6 did not alter the number of pregnancies (Bataineh et al. 2007). Distribution studies in pregnant rats given chromium(VI) or chromium(III) orally (Mertz et al. 1969) or intravenously

(Danielsson et al. 1982) and in pregnant mice given chromium(III) intraperitoneally (Iijima et al. 1983) indicated that chromium can cross the placenta after administration of either valence state. The available data on reproductive effects of chromium and its compounds are inadequate for establishing dose relationships; thus, further studies to establish the LOAEL and NOAEL values would be valuable. No dermal toxicity studies examining reproductive end points were identified; dermal studies would be useful for assessing the reproductive toxicity of chromium and compounds following dermal contact and for establishing exposure-response relationships.

Developmental Toxicity. No reliable information was located regarding developmental toxicity in humans after inhalation, oral, or dermal exposure or in animals after dermal exposure to chromium or its compounds. A study in women exposed occupationally reported that chromium can be transferred to fetuses through the placenta (Shmitova 1980), but the poor quality and reporting of this study preclude its use for drawing conclusions regarding potential developmental effects of chromium in humans. In female rats and mice, oral exposure of acute or intermediate duration to chromium(VI) compounds resulted in fetal toxicity (Elsaieed and Nada 2002; Junaid et al. 1996a, 1996b; Kanojia et al. 1996, 1998; Trivedi et al. 1989), but a NOAEL for these effects was not identified. Impaired development of the reproductive system was observed in the female offspring of mice exposed to potassium dichromate(VI) or chromium(III) chloride (Al-Hamood et al. 1998). Distribution studies in rat dams given chromium(VI) or chromium(III) intravenously (Danielsson et al. 1982) or orally (Mertz et al. 1969) and in mouse dams given chromium(III) intraperitoneally (Iijima et al. 1983) indicated that chromium can cross the placenta after administration of either valence state. No developmental effects were observed in the offspring of rats fed 1,806 mg chromium(III)/kg/day as chromium oxide for 60 days before mating and throughout the gestational period (Ivankovic and Preussmann 1975). No pharmacokinetic studies have been conducted regarding the distribution of chromium or its compounds to the fetus after inhalation or dermal exposure of the dams. Further oral developmental studies of chromium(VI) and chromium(III) in mice and other species would be useful to determine a NOAEL. These studies should include examination of developmental/neural end points. Developmental studies using inhalation exposure would be useful to determine if developmental effects are route specific. Data from oral, inhalation and dermal studies would be useful for determining dose-response relationships.

Immunotoxicity. In humans, allergic sensitization, characterized by asthma attacks and dermatitis, has been reported after occupational inhalation or occupational dermal exposure (Keskinen et al. 1980; Leroyer et al. 1998; Moller et al. 1986; Olaguibel and Basomba 1989) or dermal exposure (Burrows 1983; Engel and Calnan 1963; Engebrigtsen 1952; Eun and Marks 1990; Fregert 1975; Hansen et al. 2003;

Kaplan and Zeligman 1962; Levin et al. 1959; Nethercott et al. 1994; Newhouse 1963; Peltonen and Fraki 1983; Samitz and Shrager 1966; Wahba and Cohen 1979; Winder and Carmody 2002; Winston and Walsh 1951) to chromium compounds. Two occupational studies suggest that chromium exposure affects the leukocyte populations in the blood of workers (Boscolo et al. 1997; Mancuso 1951). Delayed anaphylactoid reaction was observed in one case (Moller et al. 1986). Dermatitis was exacerbated in sensitized individuals by oral exposure to chromium(VI) (Goitre et al. 1982; Kaaber and Veien 1977).

In rats, nonspecific disease resistance mechanisms of the lung are inhibited by inhalation exposure to chromium and its compounds (Glaser et al. 1985). Inhalation exposure of intermediate duration alters immunoglobulin levels, lymphocyte responses to antigen and lectin, and spleen weight in rats (Glaser et al. 1985), as well as alteres numbers of total recoverable cells, neutrophils, and monocytes, and percentages of pulmonary macrophages in bronchopulmonary lavage (Cohen et al. 1998). Intermediate-duration oral exposure of rats to chromium(VI) increased the proliferative response of T- and B-lymphocytes to mitogens and antigens (Snyder and Valle 1991).

There are sufficient data to determine that chromium or its compounds affect the immune system. More sensitive tests of the immune function after inhalation, oral, or dermal exposure to chromium or its compounds would be useful to determine the threshold levels for effects in humans. Studies evaluating exposure levels required to produce sensitization and elicitation of allergic responses would also provide additional information regarding threshold levels. Additional studies that explore changes in cytokine levels (Snyder et al. 1996) caused by chromium exposure should prove helpful since they may provide mechanistic information as to how chromium may affect immune function.

Neurotoxicity. Exposure of humans to high levels of airborne chromium(VI) in occupational and environmental settings produced symptoms of dizziness, headache, and weakness (Lieberman 1941). Cerebral edema was found in a case of fatal poisoning by ingestion (Kaufman et al. 1970). No studies were located describing neurotoxic effects in animals after inhalation and dermal exposure to chromium or its compounds. A 28-day drinking water study in rats reported decreased motor activity and ponderal balance, although a complete battery of neurological function tests was not conducted (Diaz-Mayans et al. 1986). Some distribution studies have detected chromium in the brain (Behari and Tandon 1980; Danielsson et al. 1982; Kaufman et al. 1970; Tandon et al. 1979). More recently, patients with 8–25-fold higher chromium blood levels that resulted from parenteral feeding did not have increased signs of somatopsychic responses (Lovrincevic et al. 1996). However, the number of patients studied was small and they were suffering from serious clinical diseases.

Since the central nervous system may be a target organ for exposure to chromium or its compounds, additional inhalation, oral, and dermal studies would be useful to corroborate the limited data and would provide useful information for populations near hazardous waste sites. More information on people (adults, children) environmentally exposed to chromium would be useful to assess its potential to effect neuro/behavioral end points.

Epidemiological and Human Dosimetry Studies. Most epidemiology studies use cohorts of occupationally exposed individuals and provide consistent data indicating that inhaled chromium can be carcinogenic (Alderson et al. 1981; Baetjer 1950b; Bidstrup 1951; Bidstrup and Case 1956; Braver et al. 1985; Cole and Rodu 2005; Crump et al. 2003; Cruz et al. 2006; Dalager et al. 1980; Davies 1979, 1984; Davies et al. 1991; EEH 1976, 1983; Enterline 1974; Fernandez-Nieto et al. 2006; Franchini et al. 1983; Frentzel-Beyme 1983; Gibb et al. 2000b; Goldbohm et al. 2006; Haguenoer et al. 1981; Hayes et al. 1979, 1989; Korallus et al. 1982; Langård and Norseth 1975; Langård and Vigander 1983; Langård et al. 1980; Machle and Gregorius 1948; Mancuso 1975, 1997a; Mancuso and Hueper 1951; Ohsaki et al. 1978; Park and Stayner 2006; Park et al. 2004;Pastides et al. 1994; PHS 1953; Rosenman and Stanbury 1996; Sassi 1956; Satoh et al. 1981; Sheffet et al. 1982; Silverstein et al. 1981; Sjogren et al. 1987; Sorahan et al. 1987; Taylor 1966) and can cause other toxic effects such as respiratory irritation, nasal septum perforation, and chrome sores on the skin (due to dermal exposure) (Bovet et al. 1977; Cohen et al. 1974; Davies et al. 1991; Gibb et al. 2000a; Gomes 1972; Hanslian et al. 1967; Keskinen et al. 1980; Kitamura et al. 2003; Kleinfeld and Rosso 1965; Lee and Goh 1988; Lieberman 1941; Letterer 1939; Lucas and Kramkowski 1975; Mancuso 1951; Meyers 1950; Novey et al. 1983; Olaguibel and Basomba 1989; Osim et al. 1999; PHS 1953; Royle 1975b; Sassi 1956; Sluis-Cremer and du Toit 1968; Sorahan et al. 1987; Taylor 1966). Results of epidemiological data are consistent with results of studies in experimental animals showing that the lung is the target organ for inhaled chromium(VI). Epidemiology studies in the chromate production industry and in chrome pigment manufacture and chrome plating have consistently shown an association with increased risk of lung cancer, but studies in other industries, such as stainless steel welding, electroplating, and ferrochromium production, have yielded inconclusive results. Exposure to chromium(VI) in these industries is associated with these effects, but the case for chromium(III) is less clear. Further studies in these industries may lead to more conclusive results. Measurements of chromium in urine and blood are useful for monitoring occupational exposure to chromium compounds. However, chromium(III) is an essential nutrient, and levels in biological fluids might be enough to mask low level exposures. One environmental epidemiology study suggested that residence near a ferrochromium plant did not pose a risk of cancer (Axelsson and Rylander 1980), but an environmental

study (which included oral exposure due to contaiminated well water) in China found that residence near an alloy plant that smelted chromium was associated with increased incidences of lung and stomach cancer (Zhang and Li 1987).

Mechanisms of Action. Numerous studies have investigated the mechanisms of celluar toxicity and genotoxicity. Toxicity appears to be related partly through reactive intermediates during intracellular reduction of chromium(VI) and oxidative reactions, and partly mediated by chromium(III), which is the final product of intracellar chromium(VI) reduction and forms deleterious complexes with critical target macromolecules (Chen and Shi 2002; Costa 2003; Costa and Klein 2006a; Ding and Shi 2002; Jeejeebhoy 1999; Levina and Lay 2005; Liu and Shi 2001; O'Brien et al. 2003; Paustenbach et al. 2003; Shrivastava et al. 2002; Zhitkovich 2005). The products of metabolic reduction of chromium(VI) (free radicals and chromium(V) and (IV)) and the newly generated chromium(III) are thought to be, in part, primarily responsible for the genotoxic effects that lead to carcinogeniticty seen in human and animal studies. The types of chromium-induced structural damage include DNA strand breaks (Aiyar et al. 1991; Bagchi et al. 2002a; Bryant et al. 2006; Casadevall et al. 1999; Ha et al. 2004; Kuykendall et al. 1996; Manning et al. 1992; Messer et al. 2006; Pattison et al. 2001; Ueno et al. 1995a), DNA-protein crosslinks (Aiyar et al. 1991; Blankenship et al. 1997; Capellmann et al. 1995; Costa et al. 1996, 1997; Kuykendall et al. 1996; Lin et al. 1992; Manning et al. 1992; Mattagajasingh and Misra 1996; Miller et al. 1991; O'Brien et al. 2005; Quievryn et al. 2001; Zhitkovich et al. 1996), DNA-DNA interstrand crosslinks (Xu et al. 1996), chromium-DNA adducts, and chromosomal aberrations (Blankenship et al. 1997; Sugiyama et al. 1986a; Umeda and Nishimura 1979; Wise et al. 1993). Results of other studies suggest that geneotoxicity of chromium is due to the formation of chromium-DNA ternary adducts, which lead to repair errors, collapsed replication forks, alterations in cellular communication, and effects on signaling pathways and cytoskelton (Ha et al. 2004), and centrosome and spindle assembly checkpoint bypass leading to chromosome instability (Holmes et al. 2006; Wise et al. 2006a). Studies on mechanisms of action of chromium are actively ongoing in the current and future literature (see Section 3.12.3, Ongoing Studies).

Biomarkers of Exposure and Effect.

Exposure. There are studies correlating chromium in urine (Gylseth et al. 1977; Iarmarcovai et al. 2005; Kilburn et al. 1990; Lindberg and Vesterberg 1983a; Lukanova et al. 1996; Medeiros et al. 2003a; McAughey et al. 1988; Minoia and Cavalleri 1988; Muttamara and Leong 2004; Mutti et al. 1985b; Sjogren et al. 1983; Stridsklev et al. 2004; Tola et al. 1977), blood (Iarmarcovai et al. 2005; Kilburn et al. 1990; Medeiros et al. 2003a; McAughey et al. 1988; Minoia and Cavalleri 1988; Muttamara and Leong

2004; Randall and Gibson 1987; Stridsklev et al. 2004; Sathwara et al. 2007), hair (Randall and Gibson 1989; Saner et al. 1984; Takagi et al. 1986), and erythrocytes (Lukanova et al. 1996; Minoia and Cavalleri 1988) to occupational exposure levels. All current methods of biological monitoring are useful primarily for occupational exposure scenarios. Since chromium is an essential element, levels of chromium compounds have to be relatively high in humans before they signify an increase due to exposure. Hair has been useful in determining chronic occupational exposure to chromium in high concentrations (Randall and Gibson 1989); the usefulness of this method for detecting prior exposures is limited to a timespan of months (Simpson and Gibson 1992). Erythrocytes (with a half-life of 120 days) can be used to monitor intermediate exposures, and blood or urine can be used to determine acute exposures (Korallus 1986a, 1986b). Occupational exposure to chromium can cause chromosomal aberrations (Koshi et al. 1984; Sarto et al. 1982; Stella et al. 1982). Therefore, chromosomal abnormalities may be useful for monitoring chromium exposure; however, other chemicals are capable of causing these effects. Chromium(VI) compounds are able to bind to macromolecules in the body and can form DNA-protein crosslinks (Coogan et al. 1991b). However, no increase in these crosslinks was observed in leukocytes from volunteers over a 240-minute time period after ingestion of chromium(VI) as potassium chromate (Kuykendall et al. 1996). The identification of chromium-protein/peptide complexes specific for chromium(VI) exposure and small enough to be excreted in the urine may be useful for biomonitoring in detecting low level exposure to populations near hazardous waste sites. As discussed in Section 3.8.1, there are a number of limitations to using urinary monitoring to assess environmental exposure to chromium (Paustenbach et al. 1997). However, urinary monitoring has the advantage of easy sample collection and is noninvasive. Mathematical models have been used to identify “excess” urinary chromium in a population exposed to low levels of chromium (Fagliano et al. 1997). Further refinement of these models as more data are collected from unexposed and exposed populations will also be useful in detecting low level exposures.

Effect. Chromosomal aberrations have been observed in workers exposed by inhalation to chromium compounds (Koshi et al. 1984; Sarto et al. 1982; Stella et al. 1982). Moreover, chromium(VI) compounds can bind to macromolecules that are excreted in the urine (Coogan et al. 1991b). The use of these techniques to detect chromosomal aberrations and chromium-macromolecular complexes would be useful in identifying populations near hazardous waste sites that would be at higher risk. In addition, the finding of increased retinol binding protein, $ß_2$-microglobulin, and brush border proteins in the urine of workers exposed to chromium may serve as an early indication of kidney damage (Franchini and Mutti 1988; Lindberg and Vesterberg 1983b; Liu et al. 1998; Mutti et al. 1985b). Additional screening for low molecular weight proteins in occupationally exposed individuals will help to determine if these proteins

can be used as reliable indicators of renal damage due to chromium exposure. Snyder et al. (1996) found no difference in mitogenic stimulation of mononuclear cells isolated from people environmentally/ occupationally exposed to chromium as compared to nonexposed individuals. However, monocytes in the exposed population had a 36% lower level of the cytokine IL-6 that is involved in antibody production. As discussed in Section 3.3, chromium induces many types of DNA lesions such as chromium-DNA complexes, DNA adducts, and DNA-protein crosslinks that are potential markers of genotoxic or cancer effects due to chromium exposure. However, only one study has attempted to utilize such end points and reported that volunteers exposed to chromium in drinking water showed no increase in protein-DNA crosslinking in blood cells (Kuykendall et al. 1996). However, further studies may show that other types of lesions induced by chromium may be more sensitive. Räsänen et al. (1991) developed an *in vitro* method to assess chromium sensitivity by measuring mononuclear leukocyte proliferation in response to chromium(III) chloride, sodium chromate(VI), and potassium chromate(VI). Additional studies would be useful to validate this method.

Absorption, Distribution, Metabolism, and Excretion. The pharmacokinetics database is substantial for human and animal exposure to chromium compounds. Chromium and its compounds can be absorbed after oral (Anderson 1981, 1986; Anderson et al. 1983; Bunker et al. 1984; DiSilvestro and Dy 2007; Donaldson and Barreras 1966; Finley et al. 1996b; Gargas et al. 1994; Kerger et al. 1997; Kuykendall et al. 1996; Paustenbach et al. 1996), inhalation (Adachi et al. 1981; Cavalleri and Minoia 1985; Gylseth et al. 1977; Langård et al. 1978; Kiilunen et al. 1983; Mancuso 1997b; Minoia and Cavalleri 1988; Randall and Gibson 1987; Suzuki et al. 1984; Tossavainen et al. 1980), and dermal (Baranowska-Dutkiewicz 1981; Brieger 1920; Corbett et al. 1997; Liden and Lundberg 1979; Mali et al. 1963; Samitz and Shrager 1966; Spruit and van Neer 1966; Wahlberg 1970; Wahlberg and Skog 1965) exposure. For the general population, oral exposure via the diet to chromium(III) is the most significant route. Occupational exposure usually involves inhalation and dermal routes. Pharmacokinetic data are generally consistent with regard to absorption, distribution, and excretion among species. Chromium(VI) compounds are absorbed more readily through cell membranes than are chromium(III) compounds (MacKenzie et al. 1958; Maruyama 1982; Witmer et al. 1989, 1991). Absorption is greater through the lungs than through the gastrointestinal tract (Baetjer et al. 1959b; Bragt and van Dura 1983; Kuykendall et al. 1996; Visek et al. 1953; Wiegand et al. 1984, 1987).

Examination of tissues taken at autopsy from occupationally and environmentally exposed people indicate widespread distribution of chromium (Brune et al. 1980; Hyodo et al. 1980; Kollmeier et al. 1990; Mancuso 1997b; Schroeder et al. 1962; Teraoka 1981). Widespread distribution of chromium has also

been found in animals after oral exposure (Kargacin et al. 1993; Witmer et al. 1989, 1991). The distribution of chromium in animals after intratracheal, parenteral, or dermal exposure is greatest in the lungs, liver, kidneys, blood, spleen, testes, and brain (Baetjer et al. 1959a; Behari and Tandon 1980; Bryson and Goodall 1983; Coogan et al. 1991b; Lim et al. 1983; Mutti et al. 1979; Tandon et al. 1979; Visek et al. 1953; Wahlberg and Skog 1965; Weber 1983). Oral exposure studies indicate that higher levels of chromium(VI) compounds are absorbed than are levels of chromium(III) compounds. Studies in humans occupationally and environmentally exposed to chromium(VI) (Casey and Hambidge 1984; Shmitova 1980) and in animals exposed to chromium(VI) or chromium(III) demonstrate the ability for chromium to cross the placenta (Mertz et al. 1969; Saxena et al. 1990a). Chromium(VI) crosses more readily than chromium(III).

There are no data to indicate that the route of exposure influences the metabolism of chromium. Regardless of the route of exposure, chromium(VI) inside the body is reduced to chromium(III) by ascorbic acid, glutathione, or by the NADPH-dependent cytochrome P450 system (Aaseth et al. 1982; Chen and Shi 2002; Costa 2003; Costa and Klein 2006a; De Flora et al. 1984, 1997; Ding and Shi 2002; Garcia and Jennette 1981; Gruber and Jennette 1978; Jeejeebhoy 1999; Levina and Lay 2005; Liu and Shi 2001; Liu et al. 1995; Mikalsen et al. 1989; O'Brien et al. 2003; Paustenbach et al. 2003; Petrilli et al. 1985, 1986a; Samitz 1970; Shrivastava et al. 2002; Suzuki and Fukuda 1990; Wiegand et al. 1984; Zhitkovich 2005).

Analysis of the urine of workers occupationally exposed to chromium(VI) indicates that chromium is excreted in the trivalent form, which is consistent with *in vivo* reduction of chromium(VI) to chromium(III) (Cavalleri and Minoia 1985; Minoia and Cavalleri 1988). Oral studies in humans and animals indicate that most of the chromium(VI) or chromium(III) ingested is excreted in the feces (Bunker et al. 1984; Donaldson and Barreras 1966; Donaldson et al. 1984; Henderson et al. 1979; Sayato et al. 1980), consistent with the poor gastrointestinal absorption of chromium. After dermal exposure of humans and animals, chromium can be found in the urine and feces (Brieger 1920; Wahlberg and Skog 1965). Chromium has been detected in hair and fingernails of the general population of several countries (Takagi et al. 1986, 1988) and in the breast milk of nursing mothers (Casey and Hambidge 1984), indicating these media as routes of excretion. Data regarding excretion after exposure of animals to chromium(VI) or chromium(III) by other routes indicated that excretion occurs rapidly, and primarily via the kidneys, once chromium(VI) is reduced (Gregus and Klaassen 1986; Yamaguchi et al. 1983). Thus, absorption, distribution, and excretion of chromium have been studied extensively. Additional studies

examining the enzymatic reduction of chromium(VI) compounds in rodents and humans would be of value in determining the potential biological impact of the reported differences in those pathways.

Comparative Toxicokinetics. Toxicokinetic data in humans, dogs, rats, mice, rabbits, and hamsters generally correlate well among species (see references above). However, exposures to chromium(VI) resulted in different organ distribution patterns between rats and mice (Kargacin et al. 1993), and the chromium levels in mouse fetal tissues were elevated over maternal blood levels, whereas in rats, these differences were not found (Saxena et al. 1990a). In addition, comparisons of human and rat hepatic microsomal ability to reduce chromium(VI) indicated differences in microsomal complexes involved (Myers and Myers 1998; Pratt and Myers 1993). Therefore, additional comparison studies among species would be useful to determine variations in the absorption, distribution, metabolism, and excretion of chromium. A PBPK model (O'Flaherty 1996; O'Flaherty et al. 2001) that has been partially validated has been developed based on rats. As described previously, the model is quite sophisticated, but additional physiological and kinetic parameters from both humans and other animal species are needed in order for the model to be employed for extrapolation across species and for use in risk assessment. Furthermore, additional metabolic data are needed with regard to insoluble chromium and its elimination and solubilization, particularly in lung tissue.

Methods for Reducing Toxic Effects. Methods for reducing the absorption of chromium from the lungs consist primarily of administering ascorbic acid or N-acetylcysteine, which enhance the reduction of chromium(VI) to chromium(III) (De Flora and Wetterhahn 1989; Suzuki and Fukuda 1990). Chromium(III) passes the alveolar lining into the bloodstream less readily than chromium(VI) and is cleared by mucociliary clearance. A study might be conducted to determine whether administration of expectorants would enhance clearance from the lungs. Oral administration of ascorbic acid to further reduce chromium(VI) to chromium(III) might further decrease bioavailability (Haddad et al. 1998; Kuykendall et al. 1996; Schonwald 2004), although this has not been proven (Leikin and Paloucek 2002; Schonwald 2004). After dermal exposure, thorough washing and ascorbic acid therapy to enhance the reduction of chromium(VI) to chromium(III) (Schonwald 2004), followed by chelation with EDTA (Nadig 1994), would greatly reduce dermal absorption. Administration of ascorbic acid has also been used to enhance the reduction of chromium(VI) to chromium(III) in plasma (Korallus et al. 1984), which would reduce the body burden of chromium because chromium(III) would bind to plasma protein and be excreted in the urine. Studies could be conducted to determine if other reducing agents would be more effective than ascorbic acid. Once inside the cell, chromium(VI) can enter many reactions resulting in reduction to various oxidation states with the generation of reactive oxygen species and radicals, all of

which may be more or less toxic than chromium(III) (De Flora and Wetterhahn 1989). Gasiorowski et al. (1997, 1998) showed that stabilizing chromium in the hexavalent oxidation state, via complexing to a ligand, decreased the mutagenicity of chromium(VI). Methods could be developed to interfere with these various reactions, but such methods may be counterproductive because they might shift one reaction to another with undesirable consequences. *In vitro* studies have indicated that vitamin E, ascorbic acid, and glutatione protected against cellular damage, including chromosomal breakage, DNA-protein crosslinks, and apoptosis (cell death) (Blankenship et al. 1997; Little et al. 1996; Sugiyama 1991; Wise et al. 1993, 2004), while vitamin B_2 enhanced the cytotoxicity and DNA single-strand breaks induced by chromium(VI) (Sugiyama 1991). Vitamin E may have scavenged radicals and/or chromium(V) during the reduction of chromium(VI) (Sugiyama 1991). Other vitamins might also be effective in mitigating chromium's effects; thus, studies on the effect of vitamins on chromium toxicity may provide additional information on the potential to reduce toxic effects. Although the administration of thyroxine has been shown to ameliorate potassium dichromate-induced acute renal failure in rats (Siegel et al. 1984), its use in humans has not been tested. Further studies are needed to assess the safety of administering thyroxine to mitigate chromium toxicity.

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

A limited amount of information is available on the toxicity of chromium in children; most of the available data come from children ingesting lethal doses of chromium(VI) (Clochesy 1984; Ellis et al. 1982; Iserson et al. 1983; Kaufman et al. 1970; Reichelderfer 1968). Studies that examine sensitive end points such as respiratory effects following inhalation exposure, or gastrointestinal, hematological, liver and kidney effects in young animals would be useful for assessing whether children will be unusually susceptible to chromium toxicity. The available animal data suggest that chromium is a developmental toxicant. As discussed in Section 3.2.2.6, the observed developmental effects include postimplantation losses, gross abnormalities, and impaired reproductive development in the offspring (Al-Hamood et al. 1998; Junaid et al. 1996a, 1996b; Kanojia et al. 1996, 1998; Trivedi et al. 1989). Data needs relating to development are discussed in detail in the Developmental Toxicity subsection above. There are some data in humans and animals that provide evidence that chromium can cross the placenta and be transferred to an infant via breast milk (Casey and Hambidge 1984; Danielsson et al. 1982; Mertz et al. 1969; Saxena et al. 1990a; Shmitova 1980). There are no data on whether chromium is stored in maternal tissues and whether these stores can be mobilized during pregnancy or lactation.

An age-related difference in the extent of gastrointestinal absorption of chromium(III) was reported in one study (Sullivan et al. 1984); it is not known if a similar relationship would exist for chromium(VI). No other information is available that evaluated potential differences between adults and children. Toxicokinetic studies examining how aging can influence the absorption, distribution, and excretion of chromium, particularly chromium(VI) would be useful in assessing children's susceptibility to chromium toxicity. There are no data to determine whether there are age-specific biomarkers of exposure or effects or any interactions with other chemicals that would be specific for children. There is very little available information on methods for reducing chromium toxic effects or body burdens; it is likely that research in adults would also be applicable to children.

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

3.12.3 Ongoing Studies

Ongoing studies pertaining to chromium toxicity have been identified and are shown in Table 3-11.

Table 3-11. Ongoing Studies on Chromium

Investigator	Study Topic	Institution	Sponsor
Avery S	Role of oxidative mechanisms in the toxicity of metals	University of Nottingham	National Institute of General Medical Sciences
Cohen M	Properties of metals may govern toxicities in the lungs	New York University School of Medicine	National Institute of General Medical Sciences
Myers C	Human lung chromium toxicity: Role of cytochrome b5	Medical College of Wisconsin	National Institute of Environmental Health Sciences
Patierno S	Chromium genotoxicity: Response and repair mechanisms	George Washington University	National Institute of Environmental Health Sciences
Stearns D	Uptake and mutagenicity of moderately soluble hexavalent chromium	Northern Arizona University	National Institute of Environmental Health Sciences
Sugden K	Oxidative DNA lesion formation from chromate exposure	University of Montana	National Institute of Environmental Health Sciences
Zhitkovich A	Biological dosimetry of hexavalent chromium	Brown University	National Institute of Environmental Health Sciences
Zhitkovich A	Genotoxicity of chromium compounds	Brown University	National Institute of Environmental Health Sciences
Zhitkovich A	Sensitivity mechanisms in chromium toxicity	Brown University	National Institute of Environmental Health Sciences

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