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Toxicological Review of Hexavalent Chromium [Cr(VI)]

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Integrated Risk Information System
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ABBREVIATIONS

ADAF	age-dependent adjustment factors
ADME	absorption, distribution, metabolism, and excretion
AIC	Akaike's information criterion
ALT	alanine aminotransferase
ALP	alkaline phosphatase
Asc	ascorbate
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMI	body mass index
BMR	benchmark response
BMDC	bone marrow-derived stem cell
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary (cell line cells)
CPHEA	Center for Public Health and Environmental Assessment
CL	confidence limit
CNS	central nervous system
Cr(III)	trivalent chromium
Cr(IV)	tetravalent chromium
Cr(V)	pentavalent chromium
Cr(VI)	hexavalent chromium
DAF	dosimetric adjustment factor
DLCO	diffusing capacity of carbon monoxide
DNA	deoxyribonucleic acid
ELF	epithelial lining fluid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV1.0	forced expiratory volume of 1 second
FVC	forced vital capacity
GD	gestation day
GGT	γ-glutamyl transferase
GI	gastrointestinal
GLP	good laboratory practices
GSD	geometric standard deviation
GSH	glutathione
GST	glutathione-S-transferase
Hb	hemoglobin
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean cell (corpuscular) volume
MEF	maximal expiratory flow
MMAD	mas median aerodynamic diameter
MN	micronuclei
MOA	mode of action
MTD	maximum tolerated dose
CPHEA	Center for Public Health and Environmental Assessment NCI National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
ORD	Office of Research and Development
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
PDC	potassium dichromate
PND	postnatal day
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
$POD_{[HED]}$	human equivalent dose POD
$POD_{[HEC]}$	human equivalent concentration POD
RBC	red blood cell, also known as erythrocyte
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RDDR	regional deposited dose ratio
RNA	ribonucleic acid
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SDD	sodium dichromate dihydrate
PK	pharmacokinetics
TSCATS	Toxic Substances Control Act Test Submissions

TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
WOS	Web of Science

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Chromium is a ubiquitous element present in soil, water, air, and food that can originate from both natural and anthropogenic sources. This toxicological review restricts its focus to hexavalent chromium compounds, which are a group of substances that contain chromium in the hexavalent (+6) oxidation state, denoted as Cr(VI). Cr(VI) compounds have many industrial applications, including pigment manufacturing, corrosion inhibition and metal finishing. Because many Cr(VI) compounds are water soluble, they are highly mobile in soil and may contaminate drinking water. Cr(VI) may be emitted into air by industries utilizing Cr(VI) compounds, and by various other sources such as the burning of fossil fuels.

The systematic review (see Appendix A for methods) conducted to support this assessment evaluated all cancer outcomes, and noncancer effects for the following potential target systems: respiratory, gastrointestinal (GI) tract, hepatic, hematologic, immune, reproductive, and developmental. For cancer and nasal effects via the inhalation route (which are well established), the systematic review focused on data that may inform the quantitative dose-response analysis.

Evidence indicates that Cr(VI) is likely to cause GI tract, liver, hematological, developmental, and lower respiratory toxicity in humans, given relevant exposure circumstances. Organ/system-specific reference values were derived for GI tract, liver, lower respiratory, and nasal effects. Evidence suggests that Cr(VI) may cause male reproductive effects and immune and hematologic toxicity in humans. Evidence is inadequate to assess whether Cr(VI) causes female reproductive toxicity in humans. The overall chronic RfD is 9×10^{-4} mg/kg-d, and the overall chronic RfC is 1×10^{-5} mg/m^3.

For cancer via the oral route of exposure, Cr(VI) is likely to be carcinogenic to the human GI tract under relevant exposure circumstances. Because a mutagenic mode of action (MOA) for Cr(VI) carcinogenicity is "sufficiently supported in (laboratory) animals" and "relevant to humans," EPA used a linear low dose extrapolation from the POD in accordance with *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Furthermore, in the absence of chemical-specific data to evaluate differences in age-specific susceptibility, increased early-life susceptibility to Cr(VI) is assumed and EPA applied ADAFs in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The total lifetime oral slope factor (OSF) for Cr(VI) is 0.5 (per mg/kg-d).

For cancer via the inhalation route of exposure, quantitative exposure-response data were evaluated, and an inhalation unit risk (IUR) was developed for human lung cancer. Similar to the oral route of exposure, linear low dose extrapolation and application of ADAFs were performed for the inhalation route of exposure. The total lifetime IUR for Cr(VI) is 2×10^{-2} (per µg Cr(VI)/m^3).

ES.1 EVIDENCE FOR HAZARDS OTHER THAN CANCER: ORAL EXPOSURE

The evidence indicates that Cr(VI) is likely to cause gastrointestinal (GI) tract, hepatic, and developmental toxicity in humans following oral ingestion (see Sections 3.2.2, 3.2.4, 3.2.5, 3.2.9). The determination that evidence indicates that Cr(VI) is likely to cause GI toxicity in humans was based on toxicology studies in rodents reporting histological effects in the GI tract. For the determination of hepatic toxicity, toxicology studies in rodents reported histological effects in the liver and serum indicators of hepatotoxicity. The determination for developmental effects was based on the observation of decreased offspring growth across most animal studies. For the hazards listed above, mechanistic evidence supported the human relevance of the effects observed in animals.

The evidence suggests that Cr(VI) may cause immune, hematologic, and male reproductive toxicity in humans (see Sections 3.2.6, 3.2.7). Male reproductive effects on sperm parameters and testosterone were observed in both human and animal studies, however most studies were considered low confidence, and effects were generally not observed in the single *high* confidence rodent study. For hematological effects, *high* confidence studies in rodents reported changes in hematological parameters that suggested a pattern consistent with regenerative microcytic hypochromic anemia, but the confidence in this judgment was diminished due to uncertainty regarding the apparent transient nature of the effects after one year. The conclusion for immune effects was primarily based on coherent evidence of effects on 1) ex vivo WBC function across human and animal studies, 2) antibody responses to T cell-dependent antigen measured in animals, and 3) reduction in host resistance to bacterial infection reported in animal studies; however, confidence in the evidence was reduced because due to primarily *low* confidence studies reporting findings that were often inconsistent across studies.

The evidence is inadequate to assess whether Cr(VI) causes female reproductive toxicity in humans (see Section 3.2.8). Although an association with female reproductive toxicity was demonstrated in a single *low* confidence epidemiology study and a series of *low* confidence animal toxicology studies, effects were not observed in *medium* or *high* confidence studies aside from a moderate decrease in maternal body weight

ES.1.1 Oral Reference Dose (RfD)

Hyperplasia in the small intestine of female B6C3F1 mice was selected as the basis for the overall chronic RfD of 9×10^{-4} mg/kg-d. A LOAEL analysis was used to derive an organ-specific point of departure (POD) for GI tract effects. Human equivalent doses (HEDs) were calculated using PBPK modeling to account for species differences and human variability in detoxification of Cr(VI) in the stomach. A composite uncertainty factor of 100 was applied. This uncertainty factor incorporated: an interspecies uncertainty (UF_A) of 3 to account for animal-to-human extrapolation (pharmacodynamic differences); an intraspecies uncertainty (UF_H) of 3 to account for variation in susceptibility across the human population, and the possibility that the available data may not be

representative of individuals who are most susceptible to the effects; and a LOAEL-to-NOAEL uncertainty (UF_L) of 10 to account for extrapolation from the LOAEL. The remaining uncertainty factors were equal to 1.

The confidence in the overall chronic RfD is high. The RfD is based on a *high* confidence chronic 2-year drinking water study by NTP (2008) that exposed rats and mice of both sexes to Cr(VI) as sodium dichromate dihydrate at drinking water concentrations from 5 mg/L to 180 mg/L (approximately 0.2 mg/kg-d to 10 mg/kg-d). Multiple *high* confidence subchronic studies also support these data (click the HAWC link for study evaluation details), and mechanistic studies support the involvement of oxidative stress in Cr(VI)-induced cytotoxicity in a variety of tissues, including the GI tract. The organ-specific RfD for the liver is also supportive of the GI tract RfD, because the GI tract and liver are exposed on first-pass following oral ingestion (so both should get the highest internal dose). While the human database for Cr(VI) induced GI toxicity was *indeterminate*, this did not warrant changing the overall confidence from *high*.

Table ES-1. Organ/system-specific RfDs and overall RfD for Cr(VI)

Hazard	Basis	osRfD mg/kg-d	Study exposure description	Confidence
Gastrointestinal system (GI tract)	Hyperplasia in small intestine of female mice	9×10^{-4}	Chronic drinking water	High
Hepatic system	Chronic inflammation in female rats	7×10^{-4}	Chronic drinking water	Medium
Developmental toxicity	Decreased F1 offspring postnatal growth	0.07	Continuous breeding	Low
Overall RfD	**GI tract effects**	**9×10^{-4}**	**Chronic drinking water**	**High**

The organ/system-specific RfD for hepatic effects was based on chronic inflammation in female F344 rats reported in NTP (2008). An organ-specific RfD of 7×10^{-4} mg/kg-d (6.69×10^{-4} rounded to 7×10^{-4}) was derived using BMD modeling. Human equivalent doses (HEDs) were calculated using pharmacokinetic modeling to account for species differences and human variability in detoxification of Cr(VI) in the stomach. A composite uncertainty factor of 100 was applied. This uncertainty factor incorporated: an interspecies uncertainty (UF_A) of 3 to account for animal-to-human extrapolation (pharmacodynamic differences); an intraspecies uncertainty (UF_H) of 3 to account for variation in susceptibility across the human population, and the possibility that the available data may not be representative of individuals who are most susceptible to the effects; and a LOAEL-to-NOAEL uncertainty (UF_L) of 10 to account for extrapolation from the LOAEL. The

remaining uncertainty factors were equal to 1. There is medium confidence in this osRfD. While it is based on a *high* confidence chronic study in rats and there are other subchronic data to support the liver endpoints (click HAWC link for study evaluation details), there were differences in the dose-response relationships between species and sexes. A lower organ-specific RfD confidence was assigned due to: 1) inconsistent responses across sex and species (e.g., histological changes were primarily seen in female rats and were less severe in male rats and mice), and 2) some uncertainty regarding the severity of the observed histological effects (specifically, the available high confidence studies did not observe a progression to more severe hepatic injury such as fibrosis or necrosis).

The organ/system-specific RfD for developmental toxicity was based on decreased F1 offspring postnatal growth from the continuous breeding study in BALBC mice (NTP, 1997). The organ-specific RfD was 0.07 mg/kg-d and was based on a NOAEL analysis. A human equivalent dose (HED) was calculated using PBPK modeling to account for species differences and human variability in detoxification of Cr(VI) in the stomach. A composite uncertainty factor of 10 was applied. This uncertainty factor incorporated: an interspecies uncertainty (UF_A) of 3 to account for animal-to-human extrapolation (pharmacodynamic differences); an intraspecies uncertainty (UF_H) of 3 to account for variation in susceptibility across the human population, and the possibility that the available data may not be representative of individuals who are most susceptible to the effects. The remaining uncertainty factors were equal to 1. There is low confidence in this osRfD. While it is based on a high confidence continuous breeding study and similar effects on decreased offspring growth observed in multiple other studies (see Section 3.2.9), this effect only occurred in high dose groups where other toxicological effects (as indicated by the lower points of departure in Table ES-2) may be occurring. A lower organ-specific RfD confidence was assigned due to: 1) the fact that no other RfDs could be derived for this endpoint from other studies due to studies being low confidence (click HAWC link for study evaluation details), and 2) the possibility that other toxicities could be affecting the animals in the high dose groups where developmental effects were observed.

Table ES-2. Summary of reference dose (RfD) derivation

Critical effect	Point of departure mg/kg-d	UF	cRfD (mg/kg-d)	Confidence
Mice (M) diffuse epithelial hyperplasia of duodenum* (NTP, 2008)	$BMDL_{10\%ER-HED}$: 0.0443	10	4.43×10^{-3}	High
Mice (F) diffuse epithelial hyperplasia of duodenum* (NTP, 2008)	**$LOAEL_{HED}$: 0.0911**	**100**	**9.11×10^{-4}**	**High**
GI tract RfD: 9.11×10^{-4} (rounded to 9×10^{-4}) mg/kg-d				
Rat (M) liver ALT (12 months) (NTP, 2008)	$BMDL_{1RD-HED}$: 0.206	10	0.0206	Medium

Critical effect	Point of departure mg/kg-d	UF	cRfD (mg/kg-d)	Confidence
Rat (M) liver ALT (3 months) (NTP, 2008)	$NOAEL_{HED}$: 0.184	30	6.13×10^{-3}	Medium
Rat (M) liver ALT (90 days) (NTP, 2007)	$LOAEL_{HED}$: 0.203	300	6.77×10^{-4}	Medium
Rat (F) liver ALT (90 days) (NTP, 2007)	$LOAEL_{HED}$: 0.190	300	6.33×10^{-4}	Medium
Rat (F) liver chronic inflammation (2 years) (NTP, 2008)	**$LOAEL_{HED}$: 0.0669**	**100**	**6.69×10^{-4}**	**Medium**
Mouse (F) liver chronic inflammation (2 years) (NTP, 2008)	$BMDL_{10\%ER\ HED}$: 0.182	10	0.0182	Medium
Rat (F) liver fatty change (2 years) (NTP, 2008)	$NOAEL_{HED}$: 0.0669	10	6.69×10^{-3}	Medium
Hepatic RfD: 6.69×10^{-4} (rounded to 7×10^{-4}) mg/kg-d				
Mouse (F) Decreased F1 postnatal growth (NTP, 1997)	**$NOAEL_{HED}$: 0.700**	**10**	**0.0700**	**Low**
Developmental RfD: 0.07 mg/kg-d				

*Duodenum: proximal subsection of the small intestine

ES.2 EVIDENCE FOR HAZARDS OTHER THAN CANCER: INHALATION EXPOSURE

As stated in the Cr(VI) IRIS Assessment Protocol (Appendix A), EPA did not re-evaluate the qualitative evidence for an association between inhalation Cr(VI) exposure and nasal effects based on EPA's 1998 evaluation of the literature and the determination that the effects of Cr(VI) on the nasal cavity have been well established [e.g., OSHA (2006) and U.S. EPA (2014c)]. Rather, the review of the evidence for nasal effects focused on identifying studies that might improve the quantitative dose-response analysis for this outcome.

EPA evaluated qualitative evidence for an association between inhalation Cr(VI) exposure and lower respiratory toxicity. EPA determined that Cr(VI) is likely to cause lower respiratory toxicity, based on evidence in six *medium* confidence animal studies examining lung cellular responses and/or histopathology. Because histopathological and cellular changes occurred together, and in combination with serum biomarkers indicating an inflammatory response, these were considered indicators of adverse responses. The human evidence for Cr(VI)-induced lower respiratory effects is limited in terms of number and confidence of studies. However, two of the available four studies provide some indication of exposure-related decrements in lung function assessed using spirometry. Mechanistic evidence supports the respiratory tract effects observed in animals.

ES.2.1 Inhalation Reference Concentration (RfC)

Effects in the nasal cavity included irritation/ulceration of the nasal mucosa or septum, perforation of the septum, and bleeding nasal septum. LOAEL analyses were used to derive the upper respiratory tract related points of departure (POD). A composite uncertainty factor of 300 was applied. This uncertainty factor incorporated: an intraspecies uncertainty factor (UF_H) of 3 to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect; a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 because this endpoint had a high incidence at the lowest concentration across multiple studies; and a subchronic-to-chronic uncertainty factor (UF_S) of 3 because data were not from chronic lifetime exposures (however the effects had a short onset time). A database deficiencies uncertainty factor (UF_D) of 3 was applied because multi-generational inhalation studies were not available in animals, human prenatal studies were rated *low* confidence, and effects of Cr(VI) differ by route of exposure due to pharmacokinetics[1] (thus, the oral database of multi-generational studies does not inform the quantitative analysis for the inhalation route). The candidate organ-specific RfC (for upper respiratory tract) was derived using data of nasal septum ulceration in humans from Gibb et al. (2000a).

For the lower respiratory tract, a candidate organ-specific RfC was derived using data of lung cellular responses and histopathological changes in rats from Glaser et al. (1990). A LOAEL analysis was used to derive most organ-specific points of departure (PODs). Human equivalent concentrations were calculated using a dosimetric adjustment factor accounting for interspecies differences in particle deposition (the regional deposited dose ratio, or RDDR). A composite uncertainty factor of 1000 was applied to the LOAEL-derived PODs (BMD-derived bronchioalveolar hyperplasia had a composite UF was 300; see Section 4.2.4). The database deficiencies uncertainty factor, UF_D, was a 3 for the same reasons specified above for the nasal osRfC. A subchronic-to-chronic uncertainty factor, UF_S, of 3 was incorporated to account for the less-than-lifetime exposure (but there was some indication in Glaser et al. (1990) that the effects were transient, and therefore a 10 was not applied). An interspecies uncertainty factor, UF_A, of 3 was applied to account for residual uncertainty in the extrapolation from laboratory animals to humans (an inhalation dosimetry factor was used to estimate a human equivalent concentration from animal data, but some pharmacodynamic uncertainty remained). A LOAEL-to-NOAEL uncertainty factor, UF_L, of 3 was applied to LOAELs because characteristics of the lung histopathological and cellular responses supported a value less than 10. UF_L of 1 was applied when BMD modeling was used (bronchioalveolar hyperplasia). An intraspecies uncertainty factor, UF_H, of 10 was applied to account for variability and uncertainty in pharmacokinetic and pharmacodynamic susceptibility within the human population (source data were only available in male inbred rats).

[1]Because Cr(VI) is detoxified in the gut on first-pass, it is possible that inhalation exposures may induce systemic effects not observed following ingestion.

The overall RfC was based on effects in the upper respiratory tract (ulceration of the nasal septum) reported by *medium* confidence studies. Effects of Cr(VI) on the nasal cavity have been well established, and this was also the most sensitive effect. It is considered protective of the other noncancer effects.

Table ES-3. Organ/system-specific RfCs and overall RfC for Cr(VI)

Hazard	Basis	osRfC mg/m^3	Study exposure description	Confidence
Respiratory (upper tract)	Ulcerated nasal septum in humans	1×10^{-5}	Occupational longitudinal study	Medium
Respiratory[a] (lower tract)	Lung cellular responses and histopathological changes in rats	1×10^{-4}	Subchronic study	Medium
Overall RfC	**Respiratory effects**	**1×10^{-5}**	Occupational longitudinal study	**Medium**

[a]Human equivalent concentrations were calculated using a dosimetric adjustment factor accounting for interspecies differences in particle deposition (the regional deposited dose ratio, or RDDR)

Table ES-4. Summary of reference concentration (RfC) derivation

Critical effect	Point of departure[a] mg/m^3	UF	cRfC mg/m^3	Confidence
Upper respiratory tract				
Ulceration of the nasal septum (Gibb et al., 2000a)	LOAEL: 3.4×10^{-3}	300	1.1×10^{-5} (rounded to 1×10^{-5})	Medium
Nasal mucosal pathology (Cohen et al., 1974)	LOAEL: 9.5×10^{-4}	300	3.2×10^{-6} (rounded to 3×10^{-6})	Medium
Ulceration of the nasal septum (Lindberg and Hedenstierna, 1983)	LOAEL: 3.3×10^{-3}	300	1.1×10^{-5} (rounded to 1×10^{-5})	Medium
Lower respiratory tract				
Histopathology: histiocytosis in rats (Glaser et al., 1990)	$LOAEL_{HEC}$: 0.133	1000	1.3×10^{-4} (rounded to 1×10^{-4})	Medium
Histopathology: bronchioalveolar hyperplasia in rats (Glaser et al., 1990)	$BMDL_{1SD}$: 0.0413	300	1.4×10^{-4} (rounded to 1×10^{-4})	Medium
Cell responses: LDH in BALF in rats (Glaser et al., 1990)	$LOAEL_{HEC}$: 0.133	1000	1.3×10^{-4} (rounded to 1×10^{-4})	Medium
Cell responses: Albumin in BALF in rats (Glaser et al., 1990)	$LOAEL_{HEC}$: 0.170	1000	1.7×10^{-4} (rounded to 2×10^{-4})	Medium
Cell responses: Total protein in BALF in rats (Glaser et al., 1990)	$LOAEL_{HEC}$: 0.133	1000	1.3×10^{-4} (rounded to 1×10^{-4})	Medium
Respiratory RfC: 1.1×10^{-5} (rounded to 1×10^{-5}) mg/m^3				

ES.3 EVIDENCE FOR HUMAN CARCINOGENICITY

Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), Cr(VI) is **likely to be carcinogenic** to humans by the oral route of exposure. The evidence of carcinogenicity to the GI tract from animal studies is *robust*, and the evidence of carcinogenicity from human studies is *slight*. There is strong supporting mechanistic evidence for Cr(VI) involvement in biological pathways contributing to carcinogenesis.

As noted in the Protocol (see Appendix A), this assessment maintains the previous determination that Cr(VI) is **carcinogenic to humans** by the inhalation route of exposure based on long-standing evidence of a causal relationship between inhalation of Cr(VI) and increased incidence of lung cancer in humans in occupational settings.

ES.4 QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK: ORAL EXPOSURE

The animal database for cancer by oral exposure consisted of a high confidence chronic 2-year drinking water bioassay which found "clear evidence of carcinogenic activity" of Cr(VI) in male and female rats and mice (NTP, 2008). These results were based on increased incidences of squamous cell neoplasms in the oral cavity of rats, and increased incidences of neoplasms in the small intestine of mice. Using these data, benchmark dose (BMD) modeling was applied to derive points of departure (PODs) for small intestinal tumors in mice and oral tumors in rats (See Section 4.3). For mice, human equivalent doses (HEDs) were calculated using PBPK modeling to account for species differences in detoxification of Cr(VI) in the stomach because tumors occurred in the small intestine (after stomach reduction to Cr(III)). For rats, HEDs were calculated using $BW^{3/4}$ scaling in accordance with U.S. EPA (2011c), because tumors occurred in the oral cavity (prior to stomach reduction to Cr(III)). In the absence of an adequately developed theory or information to develop and characterize an oral portal-of-entry dosimetric adjustment factor, application of $BW^{3/4}$ scaling is recommended (U.S. EPA, 2011c, 2005a).

The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = $0.1/BMDL_{10}$). Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/species/tumor site combination and are listed in Table ES-5. The adult-based oral slope factor for Cr(VI) is 0.3, based on tumors of the small intestine of male and female mice.

Table ES-5. Summary of oral slope factor (OSF) derivation

Critical effect	Point of departure mg/kg-d	Human equivalent dose mg/kg-d	OSF[a] $(mg/kg\text{-}d)^{-1}$	Confidence
Adenomas or carcinomas in the mouse small intestine of male mice (NTP, 2008)	$BMDL_{10\%ER}$: 1.05	0.319[b]	0.313	High

Critical effect	Point of departure mg/kg-d	Human equivalent dose mg/kg-d	OSF[a] $(mg/kg\text{-}d)^{-1}$	Confidence
Adenomas or carcinomas in the mouse small intestine of female mice (NTP, 2008)	$BMDL_{10\%ER}$: 1.03	0.316[b]	0.317	High
Squamous cell carcinoma or squamous cell papilloma in oral mucosa or tongue of male rats (NTP, 2008)	$BMDL_{10\%ER}$: 3.35	0.917[c]	0.109	High
Squamous cell carcinoma or squamous cell papilloma in oral mucosa or tongue of female rats (NTP, 2008)	$BMDL_{10\%ER}$: 2.70	0.645[c]	0.155	High
Adult-based OSF: 0.3 $(mg/kg\text{-}d)^{-1}$ (rounded from either 0.313 or 0.317) **Lifetime OSF for adenomas or carcinomas in the mouse small intestine, after application of the age-dependent adjustment factors: 0.5 $(mg/kg\text{-}d)^{-1}$ (see Section 4.3.4 for derivation)**				

[a]OSF prior to application of the age-dependent adjustment factors (see below)
[b]Estimated by PBPK modeling
[c]$BW^{3/4}$ scaling adjustment (administered dose multiplied by $(BW_A/BW_H)^{1/4}$, where BW_H = 80kg (human body weight) and BW_A (animal body weight) is set to a study-specific value.

Because a mutagenic MOA for Cr(VI) carcinogenicity (see Section 3.2.3) is "sufficiently supported in (laboratory) animals" and "relevant to humans," and as there are no chemical-specific data to evaluate the differences between adults and children, increased early-life susceptibility should be assumed. If there is early-life exposure, age-dependent adjustment factors (ADAFs) should be applied, as appropriate, in accordance with the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

The total lifetime OSF for Cr(VI) is 0.5 (per mg/kg-d). Partial oral slope factors for different age groups are provided in Section 4.3.4.

ES.5 QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK: INHALATION EXPOSURE

In 1998, the EPA IRIS Toxicological Review of Hexavalent Chromium classified Cr(VI) as a "known human carcinogen by the inhalation route of exposure" based on consistent evidence that inhaled Cr(VI) causes lung cancer in humans and supporting evidence of carcinogenicity in animals. The same conclusion has since been reached by other authoritative federal and state health agencies and international organizations and the carcinogenicity of Cr(VI) is well established for inhalation exposures (TCEQ, 2014; IPCS, 2013; NIOSH, 2013b; IARC, 2012; CalEPA, 2011; NTP, 2011; OSHA, 2006). As stated in the 2014 preliminary packages (U.S. EPA, 2014b, c) and the Systematic Review Protocol (Appendix A), the review of cancer by the inhalation route focused on data that may improve the quantitative exposure-response analysis conducted in EPA's 1998 IRIS

assessment. An overview of the literature screening for exposure-response data is contained in Section 4.4.1.

The IUR was based on an occupational cohort by Gibb et al., (2020; 2015; 2000b) of chromate production workers at a facility in Baltimore, MD. Details of the cohort are contained in Section 4.4.

Because a mutagenic MOA for Cr(VI) carcinogenicity is "sufficiently supported in (laboratory) animals" and "relevant to humans," and as there are no chemical-specific data to evaluate the differences between adults and children, increased early-life susceptibility should be assumed. If there is early-life exposure, age-dependent adjustment factors (ADAFs) should be applied, as appropriate, in accordance with the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

The total lifetime IUR for Cr(VI) is 2×10^{-2} (per µg Cr(VI)/m^3) [this value was rounded from 1.82×10^{-2} (per µg Cr(VI)/m^3)]. Partial unit risks for different age groups are provided in Section 4.4.4.

Table ES-6. Summary of inhalation unit risk (IUR) derivation

Critical effect	Basis	IUR (µg Cr(VI)/m^3)$^{-1}$	Study exposure description	Confidence
Cancer	Lung cancer (Gibb et al., 2020)	2×10^{-2}	Occupational cohort	High

ES.6 SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Susceptible populations and life stages refers to groups of people who may be at increased risk for negative health consequences following chemical exposures due to factors such as life stage, genetics, health status and disease, gender, lifestyle factors, and other co-exposures.

Populations susceptible to increased risks for negative health consequences of Cr(VI) exposure:

- Individuals with preexisting health effects that overlap with those caused by Cr(VI) exposure may be at increased risk. Health conditions that may be exacerbated by Cr(VI) exposure include gastrointestinal diseases, liver diseases, respiratory diseases, and anemia.
- Individuals with chronically high stomach pH are expected to detoxify Cr(VI) less effectively, leading to increased uptake of Cr(VI) in the gastrointestinal tract following oral exposure. High stomach pH can be caused by a number of factors, such as low gastric acid (hypochlorhydria), usage of medications to treat gastroesophageal reflux disease (GERD), and population variability.
- Individuals with genetic polymorphisms conveying deficiencies in DNA repair capacity may have increased susceptibility to Cr(VI)-induced lung cancer.

- Carriers of a mutated cystic fibrosis transmembrane conductance regulator (CFTR) allele may be at higher risk of Cr(VI)-induced cancers of the gastrointestinal tract. Suppression of the CFTR gene was shown to enhance intestinal tumorigenesis in animal models. CFTR was shown to be inactivated in mice exposed to Cr(VI). Thus, individuals with an impaired CFTR due to genetics may suffer an even further reduction in CFTR expression levels following oral exposure to Cr(VI).

Life stages susceptible to increased risks for negative health consequences of Cr(VI) exposure:

- The developmental life stage (in utero) is considered susceptible because Cr(VI) was determined to likely cause developmental toxicity in humans.
- Neonates, infants, and young toddlers less than 30 months old, which exhibit elevated stomach pH and therefore cannot effectively detoxify Cr(VI).
- For cancer effects, incorporation of age-dependent adjustment factors in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) account for early-life (<2 years) susceptibility by using a 10-fold adjustment to the slope factor.
- Elderly populations (aged 65 and older) may be at higher risk, because they exhibit some preexisting health conditions associated with aging that may be exacerbated by oral or inhalation exposure to Cr(VI).

ES.7 ORAL ABSORPTION UNCERTAINTIES AND ASSUMPTIONS APPLIED IN HAZARD IDENTIFICATION AND MODE-OF-ACTION ANALYSES

Even under controlled rodent pharmacokinetic studies, assessing the oral absorption and whole-body distribution of orally administered Cr(VI) at low doses contains uncertainty. Only total chromium can be measured in tissues in vivo. Total chromium measured in tissues following oral Cr(VI) exposure results from:

- Rapid cellular uptake of administered Cr(VI) that was absorbed into the body as Cr(VI).
- Slow cellular uptake of Cr(III) that was absorbed into the body as Cr(III), formed from administered Cr(VI) that reduced to Cr(III) extracellularly and outside of systemic circulation (e.g., gastric juices).
- Slow cellular uptake of Cr(III) that was absorbed into the body as administered Cr(VI) and reduced by other components within systemic circulation (e.g., plasma, liver, red blood cells). For example, plasma can reduce Cr(VI) extracellularly, and the resulting Cr(III) absorbed into tissues. RBCs can reduce Cr(VI) intracellularly, and the resulting Cr(III) can be released to systemic circulation (to be absorbed by other tissues) after RBCs are broken down.

- Background uptake and distribution of dietary and drinking water chromium (Cr(III) and/or Cr(VI)) not administered or controlled in the bioassay.

Additional details are provided in Section 3.1 (Pharmacokinetics) and Appendix C.1. Elevated chromium concentration in red blood cells (RBCs) is a strong indicator that Cr(VI) was absorbed in the GI tract unreduced and was not subsequently reduced by the liver during first-pass metabolism. Uptake and reduction of Cr(VI) by RBCs is rapid, and the resulting Cr(III) in red blood cells is bound to hemoglobin and/or diffuses out of the RBC slowly. Therefore, elevated RBC chromium persists longer relative to plasma chromium levels following systemic Cr(VI) absorption. Based on analyses of the RBC:plasma ratios of exposed and unexposed rodents from the NTP (2008, 2007) studies (see Appendix C.1.2), general assumptions were made when interpreting animal studies for hazard identification and MOA:

- At oral *ad libitum* doses below 1 mg/kg-d, Cr(VI) is absorbed by the GI tract, but most Cr(VI) absorbed by the GI tract is reduced to Cr(III) by the liver (and to a lesser extent, plasma and RBCs in the portal vein). At these low doses the GI tract and liver are exposed to Cr(VI), but exposure to other systems may be low and highly variable. There is high uncertainty as to whether other systemic tissues receive consistent exposure to Cr(VI) at these doses across all the studies. Therefore, inconsistent pharmacokinetic and toxicological results among studies for doses below 1 mg/kg-d are to be expected.
- At oral *ad libitum* doses greater than or equal to 1 mg/kg-d, Cr(VI) is absorbed by the GI tract, exceeds the reducing capacity of the liver, and is widely distributed to systemic tissues (e.g., kidney, lung, brain). Exposure to systemic tissues may still be highly variable, and there may be some inconsistencies in dose-response between studies.
- For oral gavage doses at any level, Cr(VI) is widely distributed to systemic tissues, and results in significantly higher internal doses than dietary and drinking water exposure. This is because the gavage route greatly condenses the timescale of an exposure, surpassing gastric reduction capacity (*ad libitum* exposures are distributed over a 24-hour period, whereas gavage occurs over a very short period).
- Injection studies (intravenous or intraperitoneal) will expose systemic tissues to significantly greater levels of Cr(VI) than oral gavage studies because there is not a first-pass effect (reduction of Cr(VI) in the stomach and liver). Following injection, there will also be (temporarily) more Cr(VI) available in the plasma prior to uptake to RBCs.

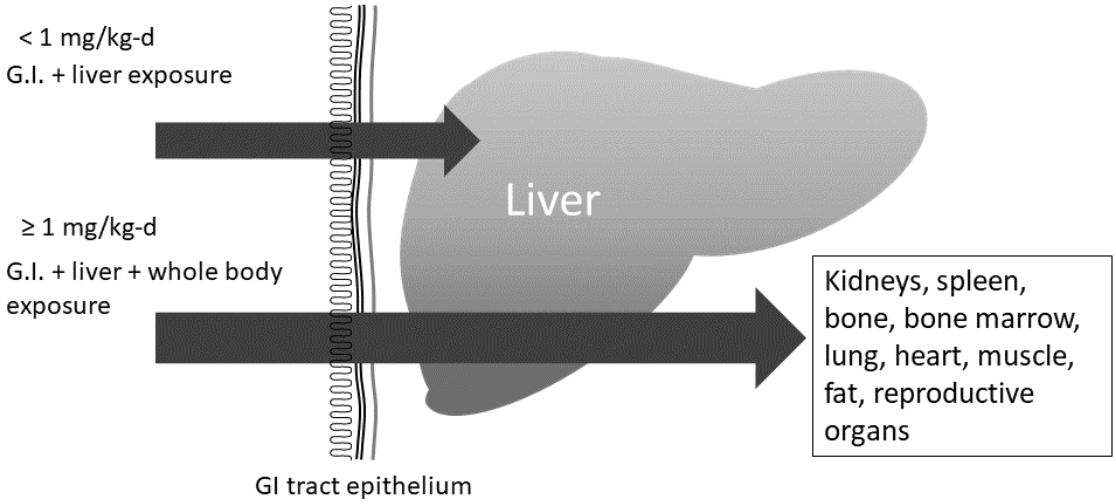


Figure ES-1-1. General assumptions regarding absorption and distribution of Cr(VI) ingested by rodents during *ad libitum* drinking water or dietary bioassays. At doses <1 mg/kg-d, it is assumed that Cr(VI) is absorbed by the small intestine, and most of the absorbed Cr(VI) is reduced by the liver. At doses ≥1 mg/kg-d, it is assumed that systemic absorption and distribution of Cr(VI) throughout the whole body will occur.

Despite uncertainties below 1 mg/kg-d, these assumptions were adequate for interpreting the current Cr(VI) database, because most studies were conducted at doses greater than 1 mg/kg-d. The 1 mg/kg-d dose level was not used as a cutoff for the inclusion of data or to make inferences about low-dose extrapolation, but instead was used to generally evaluate the uncertainties of results. For studies in which the daily oral *ad libitum* dose was much greater than 1 mg/kg-d, there is higher certainty that Cr(VI) reaches target tissues. For studies in which the daily oral *ad libitum* doses were lower than 1 mg/kg-d, there is added uncertainty when analyzing data outside of the GI or liver, because it cannot be assumed that Cr(VI) reaches other target systemic tissues at high enough doses that can induce observable effects. In general, it can be assumed that ingested Cr(VI), even at low doses, will expose at least the surface GI epithelial cells if not the liver.

1. INTRODUCTION

1.1. OVERVIEW

This Toxicological Review critically evaluates the publicly available studies on Cr(VI) in order to identify its adverse human health effects and to characterize exposure-response relationships. This assessment was prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. IRIS assessments are not regulations but provide a critical part of the scientific foundation for decisions made in EPA program and regional offices to protect public health.

This assessment updates a previous IRIS assessment of Cr(VI) (posted in 1998) that included an oral reference dose (RfD) and inhalation reference concentration (RfC) for effects other than cancer, a determination of carcinogenic potential, and inhalation unit risk (IUR) for carcinogenic effects.

As part of the initial steps in assessment development, the IRIS Program undertook scoping and initial problem-formulation activities. During scoping activities, the IRIS Program consulted with EPA program and regional offices to identify the nature of the hazard characterization needed, the most important exposure pathways, and the level of detail required to inform Agency decisions. A broad, preliminary literature survey was conducted to assist in identifying the extent of the evidence and health effects that have been studied for Cr(VI). The IRIS Program also undertook problem-formulation activities to frame the scientific questions that are a focus of this assessment. A summary of the IRIS Program's scoping and problem-formulation conclusions are contained in the 2014 preliminary packages (U.S. EPA, 2014b, c). The preliminary packages were followed by development of a Systematic Review Protocol (Appendix A), which presents detailed methods for conducting the full systematic review and dose-response analysis. As discussed in the preliminary materials and protocol, the IRIS assessment will include evaluations of the evidence relevant to all cancer outcomes and will evaluate noncancer effects for the following potential target systems: respiratory, gastrointestinal (GI) tract, hepatic, hematologic, immunological, reproductive, and developmental. For cancer and nasal irritation via the inhalation route, the systematic review will focus on data that may improve the quantitative dose-response analysis, conducted in EPA's 1998 IRIS assessment.

Appendices for additional systematic review methods and results, pharmacokinetics, dose-response modeling, and public comments are provided as Supplemental Information to this assessment (see Appendices A to F).

1.1.1. Background

Elemental chromium is a Group 6 transition metal (atomic number 24 and atomic weight 52) on the periodic table, existing in nature in the form of various oxide minerals (Anger et al., 2005). It is present in the Earth's crust and has oxidation states ranging from −2 to +6, with the +3 (trivalent) and +6 (hexavalent) states being the most common (Losi et al., 1994). Chromium in the environment can originate from both natural and anthropogenic sources (discussed in detail in Section 1.1.3) (Johnson et al., 2006; USGS, 1995; Calder, 1988; Pacyna and Nriagu, 1988). Cr(VI) compounds are used for corrosion inhibition, pigment manufacturing (including textile dyeing, printing inks, and colored glass and plastic), and metal finishing (chrome plating/electroplating) (NIOSH, 2013b; NTP, 2011). Cr(VI) has been used in wood preservatives [as chromated copper arsenate (CCA) in pressure treated wood; (ATSDR, 2012; Barnhart, 1997)]; however, this use began to decline in 2003 due to a voluntary phaseout of all residential uses of CCA pressure treated wood (Bedinger, 2015; NTP, 2011). Other uses for Cr(VI) that have been discontinued in the United States include leather tanning and corrosion inhibition within cooling systems (NIOSH, 2013b; NTP, 2011). Cr(VI) is also a byproduct of processes in the iron and steel industries (Shaw Environmental, 2006).

1.1.2. Chemical Properties

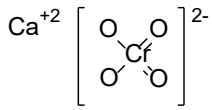
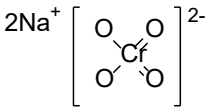
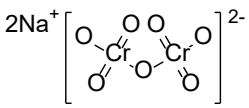
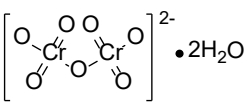
A summary of the Cr(VI) compounds encountered in the human, animal, and mechanistic studies considered pertinent to this assessment are contained in Table 1-1. This table is not an exhaustive list of all Cr(VI) species. Compounds of chromium complexed to other metals that could potentially confound the results (such as lead chromate, barium chromate, zinc chromate, copper dichromate, strontium chromate) were not included. A majority of the Cr(VI) compounds evaluated by the human, animal, and mechanistic studies relevant to this assessment are known to be water soluble. Calcium chromate was the only insoluble form, and this was used in some animal bioassays and pharmacokinetics studies. Inhalation pharmacokinetics differ between soluble and insoluble forms of Cr(VI) (OSHA, 2006) (see Section 3.1). This assessment will not make separate determinations of toxicity or carcinogenicity of soluble vs. insoluble Cr(VI) compounds because the aim is to evaluate the toxicity and carcinogenicity of Cr(VI) in all forms. Where applicable, issues related to solubility and particle size that may impact study or data interpretations are discussed during study evaluation, hazard identification, and dose-response.

Cr(VI) can exist as chromate (CrO_4^{2-}), hydrochromate ($HCrO_4^{2-}$) and dichromate ($Cr_2O_7^{2-}$) anions, whose concentrations at equilibrium depend on the metal concentration in the solution and pH. At physiological conditions (pH 7.4) and micromolar Cr(VI) concentrations, the major form of Cr(VI) is chromate and the minor form is hydrochromate, with the latter becoming a dominant form at pH≤6 (Cieślak-Golonka, 1996). Because multiple Cr(VI) compounds are discussed in this

assessment, all exposure levels were converted to Cr(VI) equivalents (see Protocol Section 8.2, Appendix A)[2].

[2]In many studies, the administered compound is stated as "sodium dichromate" ($Na_2Cr_2O_7$) when the compound is administered in aqueous solution with mass units based on sodium dichromate dihydrate ($Na_2Cr_2O_7$ $2H_2O$). Unless otherwise noted, the conversion factor for sodium dichromate dihydrate (0.349) was used to convert parent compound concentrations and doses to Cr(VI) units for studies labeled as either sodium dichromate or sodium dichromate dihydrate. Due to poor reporting, it may be unclear whether the mass per unit volume of the formulation was based on $Na_2Cr_2O_7$ $2H_2O$ or $Na_2Cr_2O_7$ (which would yield a conversion factor of 0.397). In situations where the formulation was prepared based on units of $Na_2Cr_2O_7$ mass, doses and concentrations listed in this assessment would underestimate the dose by 12%.

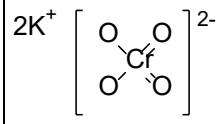
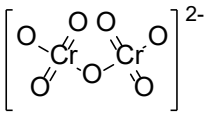
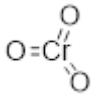
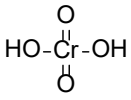
Table 1-1. Chemical identity and physicochemical properties of Cr(VI)

Name	Calcium chromate	Sodium chromate	Sodium dichromate	Sodium dichromate, dihydrate
CASRN	13765-19-0	7775-11-3	10588-01-9	7789-12-0
Synonyms	Calcium chromate(VI); calcium chrome yellow; calcium monochromate; gelbin; yellow ultramarine; chromic acid, calcium salt	Sodium chromate(VI); chromium disodium oxide; disodium chromate; rachromate; chromic acid, disodium salt; chromate of soda	Sodium dichromate(VI); sodium bichromate; dichromic acid, disodium salt; bichromate of soda	Dichromic acid, disodium salt, dihydrate
Structure				
Molecular weight	156.07	161.972	261.965	297.995
Molecular formula	$CaCrO_4$	Na_2CrO_4	$Na_2Cr_2O_7$	$Na_2Cr_2O_7 \bullet 2H_2O$
Conversion factor[a]	0.333	0.321	0.397	0.349
Melting point	1020°C (Anger et al., 2005); decomposition	794°C (Lide, 2008)	357°C (Lide, 2008)	85°C (Lide, 2008); decomposition
Density	3.12 g/cm^3 (Anger et al., 2005)	2.72 g/cm^3 (Lide, 2008)	2.52 g/cm^3 (Anger et al., 2005)	2.35 g/cm^3 (Lide, 2008)
Water solubility	4.5 g/100 g H_2O (4.3 wt%) at 0°C (Anger et al., 2005)	87.6 g/100 g H_2O at 25°C (Lide, 2008)	187 g/100 g H_2O at 25°C (Lide, 2008)	272.9 g/100 g H_2O (73.18 wt%) at 20°C (Anger et al., 2005)
Stability/ reactivity	Decomposes at 1,000°C (Lide, 2008); oxidizing agent (Lewis and Hawley, 2007)	Hygroscopic (Anger et al., 2005)	Strongly hygroscopic; decomposes above 400°C (Lide, 2008); strong oxidizing agent (Anger et al., 2005)	Very hygroscopic, deliquesces in air; strong oxidizing agent in acid solution (Lide, 2008; Anger et al., 2005)

Synonyms, structures, and molecular formulas and weights were obtained from ChemID Plus (https://chem.nlm.nih.gov/chemidplus), unless otherwise noted

[a]Mass conversion factor from parent compound to Cr(VI) units

Table 1-1. Chemical identity and physicochemical properties of Cr(VI) compounds (continued)

Name	Potassium chromate	Potassium dichromate	Chromium trioxide[b]	Chromic acid[b,c]
CASRN	7789-00-6	7778-50-9	1333-82-0	7738-94-5 (H_2CrO_4); 13530-68-2 ($H_2Cr_2O_7$)
Synonyms	Potassium chromate(VI); bipotassium chromate; dipotassium chromate; chromate of potash; tarapacaite; chromic acid, dipotassium salt	Potassium dichromate(VI); bichromate of potash; potassium bichromate; dipotassium bichromate; dipotassium dichromate; dipotassium dichromium heptaoxide; lopezite; dichromic acid dipotassium salt	Chromium(VI) oxide; hexavalent chromium oxide; chromic trioxide; chromic anhydride	Chromic(VI) acid; chromium hydroxide oxide; dichromic acid ($H_2Cr_2O_7$)
Structure				
Molecular weight	194.188	294.181	99.993	118.008 (H_2CrO_4) 218.001 ($H_2Cr_2O_7$)
Molecular formula	K_2CrO_4	$K_2Cr_2O_7$	CrO_3	H_2CrO_4; $H_2Cr_2O_7$
Conversion factor	0.268	0.353	0.520	0.441 (H_2CrO_4) 0.477 ($H_2Cr_2O_7$)
Melting point	974°C (Lide, 2008)	398°C (Lide, 2008)	197°C (Lide, 2008)	Not applicable
Density	2.73 g/cm^3 (Lide, 2008)	2.68 g/cm^3 (Lide, 2008)	2.7 g/cm^3 (Lide, 2008)	Not applicable
Water solubility	65.0 g/100 g H_2O at 25°C (Lide, 2008)	15.1 g/100 g H_2O at 25°C (Lide, 2008)	169 g/100 g H_2O at 25°C (Lide, 2008)	Not applicable
Stability/ reactivity	Nonhygroscopic (Anger et al., 2005). Strong oxidizing agent, may explode in contact with organic materials (Lewis and Hawley, 2007)	Nonhygroscopic; decomposes at 500°C (Lide, 2008; Anger et al., 2005)	Deliquescent; decomposition begins above 198°C (Anger et al., 2005); strong oxidizing agent (O'Neil et al., 2006)	Strong oxidizing agent (Anger et al., 2005)

[b]Chromic acid is formed in aqueous solution when chromium(VI) oxide is dissolved in water; it cannot be isolated as a pure compound out of solution (Anger et al., 2005; Page and Loar, 2004). The term chromic acid is sometimes used to reference chromium(VI) oxide; however, it should be noted that there is a structural difference between the anhydrous substance chromium(VI) oxide and the aqueous chromic acid that forms when the oxide is dissolved in water.

[c]Chromic acid exists in solution as both H_2CrO_4 and $H_2Cr_2O_7$ (Anger et al., 2005; Page and Loar, 2004; Cotton et al., 1999). H_2CrO_4 is the main species in basic solutions (pH > 6) while $H_2Cr_2O_7$ is the main species in strongly acidic solutions (pH < 1) (Anger et al., 2005; Page and Loar, 2004; Cotton et al., 1999). Both species are present in equilibrium in solutions that have a pH value between 2 and 6 (Anger et al., 2005; Page and Loar, 2004; Cotton et al., 1999).

1.1.3. Sources, Production, and Use

1.1.3.1. *Soil*

The EPA Toxics Release Inventory (TRI) estimates approximately 53 million pounds of chromium and chromium compounds were released to the environment via land releases (such as landfills, land treatment, and surface impoundments, excluding underground injections) (U.S. EPA, 2018). Sources of chromium releases into soil include the disposal of commercial products that contain chromium, coal fly ash and bottom fly ash from electric utilities and other industries, solid wastes from metal manufacturing and chrome-plating facilities, chromate production waste, agricultural and food wastes, leather tannery waste, and cooling tower water containing rust inhibitors (Oregon DEQ, 2014; ATSDR, 2012; U.S. EPA, 2011b; Pellerin and Booker, 2000; Burke et al., 1991; Nriagu and Pacyna, 1988). Air deposition to soil from combustion processes also occurs.

Cr(III) in soil may be present predominantly as chromium hydroxide ($Cr(OH)_3$) or chromium oxide (Cr_2O_3) (Apte et al., 2006; Kim and Dixon, 2002). These Cr(III) forms have low solubility and reactivity. Cr(VI) may exist in soil as chromate (CrO_4^{-2}), chromic acid ($HCrO_4^-$), dichromate ($Cr_2O_7^{-2}$), and chromate salts ($BaCrO_4$, $CaCrO_4$, $PbCrO_4$, $ZnCrO_4$) (ATSDR, 2012; Apte et al., 2006; Kim and Dixon, 2002). Conversion of Cr(VI) to Cr(III) may occur in the environment under reducing conditions (by ferrous iron, sulfides, and organic matter), while conversion of Cr(III) to Cr(VI) may occur under oxidizing conditions (by manganese oxide minerals) (Hausladen et al., 2018; 2017; McClain et al., 2017; Jardine et al., 2011; Cummings et al., 2007; Oze et al., 2007; 2004; Kim and Dixon, 2002; Fendorf et al., 2000; 1995). Fire-induced oxidation of Cr(III)-substituted iron oxides in soils may also occur during wildfires (Burton et al., 2019).

Most Cr(III) compounds are insoluble in water and immobile in soils (which helps inhibit oxidation), while most Cr(VI) compounds are readily soluble in water and highly mobile and bioavailable (Fendorf et al., 2000; Fendorf, 1995). In addition to being stabilized by low solubility and mobility, Cr(III) compounds are more thermodynamically stable than Cr(VI) compounds under most pH values encountered in the environment (Fendorf, 1995). And therefore, the predominant direction of chromium transformation in the environment is Cr(VI) →Cr(III). See Figure 1-1.

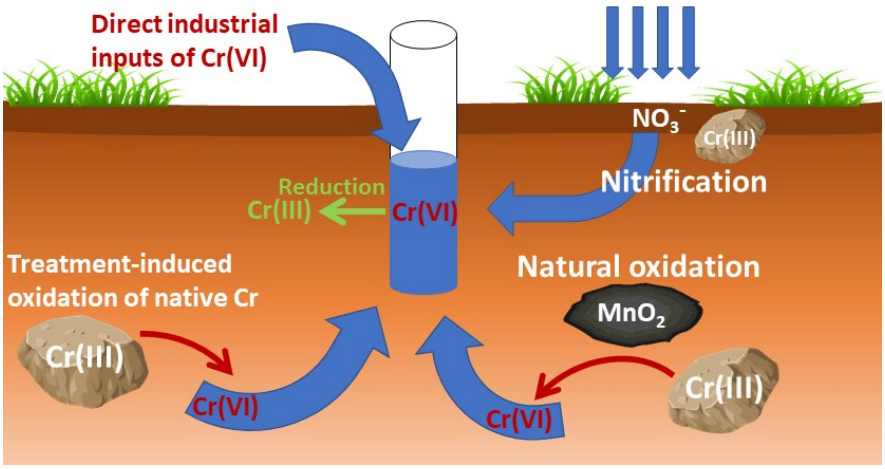


Figure 1-1. Sources of Cr(VI) in soil and groundwater. Adapted from (Hausladen et al., 2018).

1.1.3.2. *Water*

The EPA Toxics Release Inventory (TRI) estimates approximately 66,000 pounds of chromium and chromium compounds were released to the environment via surface water discharges, and 315,000 pounds were discharged for wastewater treatment in 2019 (U.S. EPA, 2018). Data from USEPA's Discharge Monitoring Report (DMR) estimates that approximately 90,000 pounds of Cr(VI) was discharged in 2020 (U.S. EPA, 2014a). Most chromium released into water from anthropogenic sources is ultimately deposited in sediment. Chromium in the aqueous phase is mostly present as soluble Cr(VI) or as soluble Cr(III) complexes. Reduction of Cr(VI) to Cr(III) can occur in the presence of reducing agents (e.g., organic matter, hydrogen sulfide, sulfur, iron sulfide, ammonium, nitrate). The reduction half-life of Cr(VI) in water can be rapid (ranging from instantaneously to a few days) when reducing agents are present under anaerobic conditions, but can extend from 4–140 days in water with soil and organic sediment (Saleh et al., 1989). Oxidation of Cr(III) to Cr(VI) can also occur within aquifers and water treatment systems (Chebeir and Liu, 2016; U.S. EPA, 1986a). The ratio of Cr(VI) to Cr(III) has been measured to be higher in groundwater than in surface water (Frey et al., 2004). Oxidizing conditions within soil, as well as the natural Cr(VI) content of soil and rocks, also affect Cr(VI) content of water (Vengosh et al., 2016). Above-average groundwater levels of Cr(VI) have been reported in several areas in the Western US (U.S. EPA, 2014d).

1.1.3.3. *Air*

Approximately 222,840 pounds of chromium and chromium compounds were released from fugitive and point sources into air from reporting facilities in 2020 (U.S. EPA, 2021b). Based on data from the 2017 EPA National Emissions Inventory (NEI), approximately 64,208 pounds of Cr(VI), 1,392 pounds of chromic (VI) acid, 86 pounds of Chromium (VI) Trioxide, and 373,891 pounds of chromium (III) were released into the air nationwide (U.S. EPA, 2021a). The NEI includes

additional emissions sources not reported under TRI (i.e., mobile sources). Atmospheric chromium particles resulting from industrial emissions have been reported to have a mass mean aerodynamic diameter (MMAD) of less than 10 μm, were found to remain airborne for 7–10 days, and were subject to long-range transport (Kimbrough et al., 1999). Atmospheric particulate matter is deposited on land and water via wet and dry deposition, and metals may deposit at a higher rate in urban areas relative to rural and remote locations (Schroeder et al., 1987). Transport of chromium from water to the atmosphere is possible via transport in windblown seasalt sprays (Nriagu, 1989). Major atmospheric chromium emissions from anthropogenic sources in the United States are outlined in Table 1-2.

Table 1-2. Major anthropogenic sources of atmospheric chromium in the United States [adapted from ATSDR (2012)]

Combustion of coal and oil	Utility industry cooling towers
Ferrochromium production	Chemical manufacturing cooling towers
Chromium chemical manufacturing	Petroleum refining cooling towers
Chrome plating	Glass manufacturing cooling towers
Chrome ore refining	Primary metal cooling towers
Refractory production	Comfort cooling towers
Cement production	Textile manufacturing cooling towers
Specialty/steel production	Tobacco cooling towers
Sewage sludge incineration	Tire and rubber cooling towers
Municipal refuse incineration	

Data of annual Cr(VI) emissions in the US can be obtained from the EPA National Emissions Inventory (U.S. EPA, 2016a).

Depending on the emission source, different forms of Cr(VI) may be emitted (i.e., Cr(VI) acid mists/dissolved aerosols, and Cr(VI) dusts). While information is limited regarding non-occupational inhalation exposures to chromic acid mists for the general U.S. population, residents of fence-line communities may be exposed to multiple forms of Cr(VI) (OAQPS, 2012). Chrome-plating facilities and private residencies may exist in close proximity in mixed land use communities (CARB, 2004; CalEPA, 2003). Chromium trioxide (CrO_3) is the acidic anhydride of chromic acid (H_2CrO_4). Chromic acid in mists or vapors dehydrates to CrO_3 upon evaporation, and some CrO_3 may convert to H_2CrO_4 in moist environments (including the respiratory tract).

1.1.4. Environmental Occurrence

Measured concentrations of Cr(VI) in the United States and selected U.S. locations are listed in Table 1-3. The mean soil concentration of total chromium in the United States is approximately 36 mg/kg, and the ratio of Cr(VI) to Cr(III) depends on several factors (such as soil pH). Nationwide data for speciated chromium are unavailable, although some site-specific soil concentrations of Cr(VI) have been reported. For example, soil Cr(VI) concentrations in Montana were mostly below the limit of detection of 0.29 mg/kg (Hydrometrics, 2013). Cr(VI) concentrations near industrial

facilities in Portland, Oregon were typically below 1 mg/kg but were measured as high as 3 mg/kg (Oregon DEQ, 2016a, c).

Public water system data from EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3)[3], includes both groundwater and surface water sources (U.S. EPA, 2014d). Mean Cr(VI) concentrations in public water systems averaged approximately 0.48 µg/L for large systems (U.S. EPA, 2014d). There was wide variability by region (Figure 1-2), and a maximum concentration of 97.4 µg/L.

Ambient air concentrations of Cr(VI) in the United States typically range from 0.01 to 0.05 ng/m^3 (U.S. EPA, 2016d), but have been measured at values above 1 ng/m^3 for urban and industrial areas (Oregon DEQ, 2016b; Huang et al., 2014). Historically, Cr(VI) concentrations measured in ambient air downwind of industrial facilities emitting Cr(VI) (such as chrome platers) have been found to be highly correlated with concentrations measured at the facilities (SCAQMD, 2016). Between May 2001-May 2002, residential air near chrome-plating facilities in San Diego, CA were measured up to 22.0 ng/m^3 Cr(VI) (CalEPA, 2004, 2003).

Table 1-3. Estimated environmental concentrations of chromium in the United States

Media and location	Units	Mean	Max	Reference
Ambient air, US	ng/m^3 Cr(VI)	0.037	0.5	EPA Air Monitoring Archive for Hazardous Air Pollutants (U.S. EPA, 2016b) for 2015
Drinking water, US	µg/L Cr(VI)	0.48	97.4	EPA Third Unregulated Contaminant Monitoring Rule (UCMR3) (U.S. EPA, 2014d)
Surface soil, US	mg/kg total chromium	36	4,120	Data from USGS (Smith et al., 2013)

[3]Cr(VI) was among 30 contaminants selected for monitoring at public water systems (PWS) for the Third Unregulated Contaminant Monitoring Rule (UCMR3) between 2013 and 2015. A PWS is a network of pipes and conveyances constructed to provide water for human consumption (U.S. EPA, 2006a, b). Small systems, serving 10,000 or fewer people, account for more than 97% of the total number of PWSs, while large systems, serving more than 10,000 people, account for the remaining 3% (U.S. EPA, 2006a, b). A majority of the U.S. population is served by large PWSs (nearly 90% (U.S. EPA, 2006a, b)), and all of them (approximately 4,200) were tested under UCMR3. For small water systems, approximately 800 systems were randomly selected and used as a representative sample (U.S. EPA, 2012c). Small water systems were omitted from analyses presented in this section. Cr(VI) was selected for the UCMR3 cycle, and was not selected for monitoring for the UCMR4 or UCMR5 cycles.

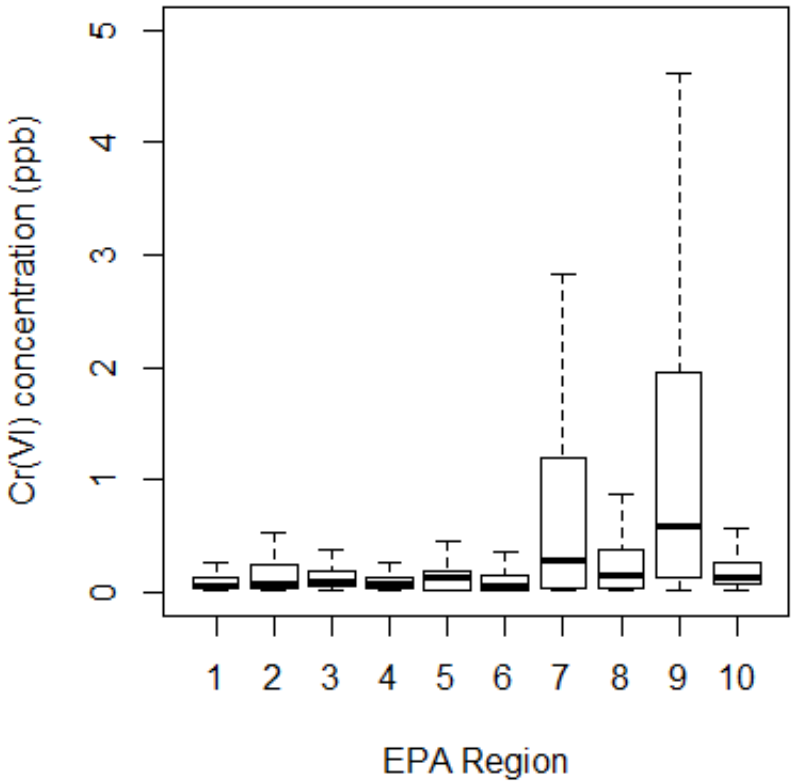


Figure 1-2. Drinking water chromium (VI) concentrations in the United States by EPA region.[4] Boxplots are based on the average values of samples of large public water systems within the region, from EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) (**U.S. EPA, 2014d**). Boxes represent interquartile ranges. Whiskers are 1.5x the interquartile range away from the 25th/75th percentiles.

1.1.5. Potential for Human Exposure

1.1.5.1. *General population*

General population exposures to Cr(VI) occur via inhalation of ambient air, ingestion of water or food, and non-dietary ingestion of soil or dust. Most human exposure to total chromium

[4]Region 1 - CT, ME, MA, NH, RI, and VT
Region 2 - NJ, NY, Puerto Rico, and the U.S. Virgin Islands
Region 3 - DE, DC, MD, PA, VA, WV and 7 federally recognized tribes
Region 4 - AL, FL, GA, KY, MS, NC, SC, and TN
Region 5 - IL, IN, MI, MN, OH, and WI
Region 6 - AR, LA, NM, OK, and TX
Region 7 - IA, KS, MO, and NE
Region 8 - CO, MT, ND, SD, UT, and WY
Region 9 - AZ, CA, HI, NV, American Samoa, Commonwealth of the Northern Mariana Islands, Federated States of Micronesia, Guam, Marshall Islands, and Republic of Palau
Region 10 - AK, ID, OR, WA and 271 native tribes.

(sum of Cr(VI) and Cr(III)) is from dietary intake of Cr(III) that is naturally present in foods (Wisconsin DHS, 2010). Cr(III) is generally understood to be essential to normal glucose, protein, and fat metabolism and is thus an element with an Adequate Intake (AI)[5] values (IOM, 2011), although no Recommended Daily Allowance (RDA) has been established due to insufficient evidence to establish a level of Cr(III) that is necessary for human health (NIH, 2017; Vincent, 2017; Vincent, 2013; Stearns, 2000). Dermal exposure may also occur during the use of consumer products that contain chromium, such as some metals and wood or leather treated with chromium-containing compounds (ATSDR, 2012; NTP, 2011). Concentrations that non-occupationally exposed humans may be exposed to were provided earlier in Table 1-3.

Quantifying the non-dietary exposure to Cr(VI) via soil ingestion (hand-to-mouth contact and pica behavior in children) is uncertain due to limited data on chromium speciation in soil. As noted earlier, the Cr(VI)/Cr(III) concentration ratio in soil can vary due to factors such as soil pH and mineral content, and no nationwide data on soil Cr(VI) currently exist. Quantifying dietary exposure to Cr(VI) via food ingestion is also uncertain due to limited data on speciation in food. Typical total chromium (sum of Cr(VI) and Cr(III)) levels in most foods have been reported to range from <10 to 1,300 µg/kg, with the highest concentrations being found in meat, fish, fruits, and vegetables (WHO, 2003). Dietary total chromium intake in the general U.S. population has been estimated to range from 0.293–0.867 µg/kg-day (ATSDR, 2012; Moschandreas et al., 2002). It is possible that a fraction of this intake is in the form of Cr(VI) (Hamilton et al., 2018). Mathebula et al. (2017) found that 33–73% of total chromium in bread may exist as Cr(VI) (at concentrations between 19–64 µg/kg), and that oxidation of Cr(III) to Cr(VI) can occur from toasting. That study also detected Cr(VI) in breakfast cereals at concentrations between 41–470 µg/kg. Soares et al. (2010) estimated that 12% of total chromium in bread was hexavalent. However, nationwide data for Cr(VI) content in food is limited.

According to data collected between 2013 and 2015 under EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3), Cr(VI) has been reported above the minimum reporting limit (0.03 µg/L) by approximately 90% of public water systems in the United States (U.S. EPA, 2014d). More detailed concentration data for Cr(VI) in large U.S. water systems are provided in Appendix C.4.

The general population may be exposed to Cr(VI) in air but will likely receive a lower inhaled dose when compared to the oral ingestion pathway. A 70 kg individual drinking 2L/day water containing 0.5 µg/L Cr(VI) (Table 1-3) will ingest a dose of 1.4×10^{-5} mg/kg-d Cr(VI). A 70 kg individual with a respiratory rate of 20 m^3/day inhaling air containing 4×10^{-5} µg/m^3 Cr(VI) (Table 1-3) will inhale Cr(VI) at a body weight-normalized rate of 1.1×10^{-8} mg/kg-d. Both air and water concentrations may vary from the approximate mean values by a factor of 100 in extreme cases (see Appendix C.4). As a result, it is possible for the inhaled dose to be comparable to the ingested dose for people living in an area with low Cr(VI) in water and high Cr(VI) in air.

[5]Adequate intakes of chromium for adult males and females are 35 µg/day and 25 µg/day, respectively.

Inhalation of Cr(VI) in water droplets during showering can also occur. Since Cr(VI) cannot volatilize, and because Cr(VI) compounds are typically water soluble, the metal will exist only in water droplets and aerosols. An analysis of this exposure pathway was performed by California EPA, and determined that a 70-kg adult breathing 20 m^3 of air per day, taking a 10-minute shower would inhale 27 mg of liquid water per shower (3.86×10^{-7} L/kg-d) (CalEPA, 2011). Assuming water contains 0.5 µg/L Cr(VI) yields an inhaled dose of 1.9×10^{-10} mg/kg-d, which is five orders of magnitude less than the dose resulting from 2 L/day water ingestion at the same Cr(VI) concentration (1.4×10^{-5} mg/kg-d).

Humans may be exposed via inhalation and incidental ingestion of house dust. A study of house dust in areas with no known soil contamination by Cr(VI) in New Jersey measured a mean Cr(VI) surface loading of 10 µg/m^2 (maximum of 169.3 µg/m^2), and mean Cr(VI) concentration of 4.6 µg/g (maximum of 56.6 µg/g) (Stern et al., 2010). Nationwide data of Cr(VI) in house dust are unavailable.

1.1.5.2. *Occupational exposure*

Occupational exposures to Cr(VI) occur primarily via inhalation or dermal contact (NIOSH, 2013b) and typically exceed those of non-occupational exposures (NTP, 2011). Workers can potentially inhale Cr(VI) during its processing or manufacture and when working with mixtures containing the chemical or chemical precursors. Dermal exposures may potentially result from the splashing or spilling of chromium-containing materials that contact the skin or from contact with construction materials containing Portland cement (due to a Cr(VI) impurity) (NIOSH, 2013b). Portal-of-entry sites may be exposed via hand-to-mouth contact and hand-to-nose contact (OSHA, 2006), and the extent of these transfers depends on the industry, exposure matrix, and workplace hygiene practices (Cohen et al., 1974). Industries that may have workers who are in contact with Cr(VI)-containing materials include stainless-steel welding, painting, electroplating, steel mill, iron and steel foundries, wood preserving, and occupations that produce paints, coatings, inks, plastic colorants, chromium catalyst, and other chemicals (such as chromium dioxide and chromium sulfate) (NIOSH, 2013b). Other industries with limited potential exposures to Cr(VI) compounds include textile dyeing, glass production, printing, leather tanning, brick production, woodworking, solid waste incineration, oil and gas well drilling, construction and Portland cement production (NIOSH, 2013b; NTP, 2011). Table 1-4 provides a list of industries that are potential sources of chromium exposure.

Table 1-4. Industries and occupations that may be sources of chromium exposure

Group 1: Industry sectors where majority of occupational exposures occur to hexavalent chromium	Group 2: Industry sectors with limited potential for occupational exposure to hexavalent chromium
Electroplating	Chromium Dioxide Producers
Welding	Chromium Dye Producers
Painting	Chromium Sulfate Producers
Producers of Chromates and Related Chemicals from Chromite Ore	Chemical Distributors
Chromate Pigment Production	Textile Dyeing
Chromated Copper Arsenate Producers	Producers of Colored Glass
Chromium Catalyst Production	Printing
Paint and Coatings Production	Leather Tanning
Printing Ink Producers	Chromium Catalyst Users
Plastic Colorant Producers and Users	Producers of Refractory Brick
Plating Mixture Production	Woodworking
Wood Preserving	Solid Waste Incineration
Chromium Metal Production	Oil and Gas Well Drilling
Steel Mills	Portland Cement Producers
Iron and Steel Foundries	Non-Ferrous Superalloy Producers and Uses
	Construction
	Producers of Pre-Case Concrete Products

Source: Analysis performed by OSHA (Shaw Environmental, 2006)

1.2. SUMMARY OF ASSESSMENT METHODS

The methods used to conduct this assessment, including systematic review procedures and approaches for dose-response analysis, are summarized in the remainder of this section. A detailed description of these methods is provided in the preliminary materials released in 2014 (U.S. EPA, 2014b, c) and in the Systematic Review Protocol for Cr(VI) in Appendix A.

1.2.1. Literature Search and Screening

Literature search strategies were developed using key terms and words related to the PECO criteria and potentially relevant supplemental material. Relevant subject headings and text-words were crafted into a search strategy that was designed to maximize the sensitivity and specificity of the search results. The search strategy was run, and the results were assessed to ensure that all previously identified relevant primary studies were retrieved in the search. Because each database has its own search architecture, the resulting search strategy was tailored to account for the unique search functionality of each database.

The following databases were searched:

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)

- Toxline (National Library of Medicine)[6]

Searches were not restricted by publication date, and no language restrictions were applied. Web of Science results were limited using the research areas filter. All Web of Science research areas identified in the search results were prioritized by a technical advisor as high priority (e.g., toxicology), low priority (e.g., chemistry), and not relevant (e.g., forestry). Literature searches were conducted in bibliographic databases as described in Appendix B and uploaded to EPA's Health and Environmental Research Online (HERO) database.[7]

Additional relevant literature not found through database searching was sought by:

- Manually searching citations from review articles and studies considered to meet PECO criteria after screening ("included" studies).
- Searches of gray literature, including primary studies that are not indexed in databases of peer-reviewed literature (e.g., technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; working papers from research groups or committees; and white papers), or other nontypical searches. Gray literature is typically identified by searching the EPA Chemical Dashboard (https://comptox.epa.gov/dashboard) during problem formulation, by engaging with technical experts, and during solicitation of Agency, interagency, and public comment at multiple steps in the IRIS process.
- "Backward" searches (to identify articles cited by included studies, reviews, or prior assessments by other agencies).

The results returned (i.e., the number of "hits" from each electronic database or other literature source), including the results of any literature search updates, are documented in the literature flow diagrams, which also reflect the literature screening decisions (see Section 2.1).

The IRIS Program takes extra steps to ensure identification of pertinent studies by (1) encouraging the scientific community and the public to identify additional studies and ongoing research; (2) searching for publicly available data submitted under the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act; and (3) considering late-breaking studies that would impact the credibility of the conclusions, even during the review process. Studies identified after peer review begins will only be considered for inclusion if they meet the PECO criteria and may fundamentally alter the assessment's conclusions.

1.2.2. Evaluation of Individual Studies

The detailed approaches used for the evaluation of epidemiologic and animal toxicology studies used in the Cr(VI) assessment are provided in the protocol (Appendix A). The general approach for evaluating health effect studies meeting PECO criteria is the same for epidemiology

[6]TOXLINE was phased out in December 2019 and integrated into other NLM resources.
[7]Health and Environmental Research Online: https://hero.epa.gov/hero/.

and animal toxicology studies although the specifics of applying the approach differ; thus, they are described in detail in protocol Sections 6.2 and 6.3, respectively, in Appendix A.

- The key concerns for the review of epidemiology and animal toxicology studies are potential bias (factors that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect; low sensitivity is a bias towards the null when an effect exists). In terms of the process for evaluating individual studies, two or more reviewers independently arrive at judgments regarding the reliability of the study results (reflected as study confidence determinations; see below) with regard to each outcome or outcome grouping of interest; thus, different judgments are possible for different outcomes within the same study. The results of these reviews are tracked within EPA's version of the Health Assessment Workplace Collaboration (HAWC).
- To develop these judgments, each reviewer assigns a category of *good*, *adequate*, *deficient* (or *not reported*, which generally carries the same functional interpretation as *deficient*), or *critically deficient* (listed from best to worst methodological conduct; see Section 6.1 of the protocol in Appendix A for definitions) to each evaluation domain representing the different characteristics of the study methods that were evaluated based on the criteria outlined in HAWC. Reviewers assigning categories to each domain are guided by core and prompting questions as well as additional considerations specific to Cr(VI) or the outcome of interest. Exposure-specific considerations in epidemiology studies are described in Section 6.2. Briefly, air concentration measurements were preferred to biomarker measurements. Studies in which human exposure was quantified by measurements of total chromium in urine, blood, plasma, or erythrocytes were considered for determination of hazard only if conducted in workers with known occupational exposure to Cr(VI).

Once all evaluation domains were evaluated, the identified strengths and limitations are considered as a whole by the reviewers in order to reach a final study confidence classification:

- *High* confidence: No notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology.
- *Medium* confidence: Possible deficiencies or concerns were noted, but the limitations are unlikely to be of a notable degree or to have a notable impact on the results.
- *Low* confidence: Deficiencies or concerns were noted, and the potential for bias or inadequate sensitivity could have a significant impact on the study results or their interpretation. *Low* confidence results were given less weight compared to *high* or *medium* confidence results during evidence synthesis and integration (see Sections 1.2.4 and 1.2.5).
- *Uninformative*: Serious flaw(s) were identified that make the study results unusable. *Uninformative* studies were not considered further, except to highlight possible research gaps.

Using the HAWC platform (and conflict resolution by an additional reviewer, as needed), the reviewers reached a consensus judgment regarding each evaluation domain and overall (confidence) determination. The specific limitations identified during study evaluation were carried forward to inform the synthesis (Section 1.2.4) within each body of evidence for a given health effect (i.e., study confidence determinations were not used to inform judgments in isolation). Additional details regarding study evaluation are provided in Sections 6.1–6.5 of the protocol (Appendix A).

1.2.3. Data Extraction

The detailed data extraction approach is provided in Section 8 and Appendix B of the protocol (Appendix A). Animal data extraction and content management were carried out using HAWC, while human epidemiology data were summarized in tabular format in the assessment and appendices. In general, studies evaluated as being *uninformative* were not considered further and study details are not provided. In addition, study details and results for outcomes determined to be less relevant during PECO refinement were not extracted or were only partially extracted (Appendix A). The same was typically true for *low* confidence studies where a number of *medium* and *high* confidence studies were available, unless the *low* confidence studies included study designs lacking in the higher confidence studies (e.g., testing lower exposure levels, or susceptible populations or life stages). The level of extraction for specific outcomes within a study may differ (i.e., ranging from a narrative to full extraction of dose-response effect size information). Data extraction was performed by one member of the evaluation team and checked by at least one other member.

For animal data already extracted to evidence tables released in 2014 (U.S. EPA, 2014b), data extraction procedures depended on data type (e.g., dichotomous, continuous, or qualitative). For human data already extracted to evidence tables released in 2014 (U.S. EPA, 2014c), data extraction procedures depended on the quality of the study and the study design. A detailed discussion of the methods used for data extraction are provided in Section 8 of the protocol (Appendix A). Extracted data are available in HAWC and are also summarized in tabular or graphical form in the hazard identification and dose-response sections.

1.2.4. Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes (see Protocol Sections 9 and 10, Appendix A for full details). For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each synthesis providing a summary discussion of the available evidence that addresses considerations regarding causation that are adapted from Hill (1965). Mechanistic evidence and other supplemental information is also

synthesized to address key science issues and/or to help inform key decisions regarding the human and animal evidence.

The syntheses focus on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of available studies. The human and animal health effects evidence syntheses are based primarily on studies of *high* and *medium* confidence. *Low* confidence studies may be used if few or no studies with higher confidence are available to help evaluate consistency, or if the study designs of the *low* confidence studies address notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect. If *low* confidence studies are used, then a careful examination of risk of bias and sensitivity with potential impacts on the evidence synthesis conclusions is included in the narrative. The synthesis of mechanistic evidence and other supplemental information informs the integration of health effects evidence for both hazard identification (i.e., biological plausibility of the available human or animal evidence; inferences regarding human relevance, or the identification of susceptible populations and life stages across the human and animal evidence) and dose-response evaluation.

For each assessed health effect, following the evidence syntheses, integrated judgments are drawn across all lines of evidence. During evidence integration, a structured and documented process was used, as follows:

- Building from the separate syntheses of the human and animal evidence, the strength of the evidence from the available human and animal health effect studies was summarized in parallel, but separately, using a structured evaluation of an adapted set of considerations first introduced by Bradford Hill (Hill, 1965). These summaries incorporate the relevant mechanistic evidence (or MOA understanding) that informs the biological plausibility and coherence within the available human or animal health effect studies.
- The strength of the animal and human evidence was considered together in light of inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal and mechanistic evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human relevance of animal findings is assumed.
- A summary judgment is drawn as to whether the available evidence base for each potential human health effect as a whole provides sufficient evidence to indicate that Cr(VI) exposure has the potential to cause the health effect in humans; insufficient evidence to assess whether Cr(VI) exposure has the potential to cause the health effect in humans; or, in rare instances, sufficient evidence that a hazard is unlikely.

The decision points within the structured evidence integration process are summarized in an evidence profile table for each assessed health effect.

Outlines of the major endpoints assessed within each health effect domain are listed below in Table 1-5.

Table 1-5. Endpoint grouping categories

Relevant human health effect category	Examples of endpoints included[a]	Notes
General toxicity	• Body weight (not maternal or pup weights, or weights after developmental-only exposure) • Mortality, survival, or LD_{50}s • Growth curve • Clinical observations (non-behavioral)	• Clinical chemistry endpoints are under hepatic or hematologic effects • Maternal or pup body-weight endpoints are under developmental effects • Pathology (including gross lesions) is organ specific
Hepatic effects[b]	• Liver weight and histopathology • Serum or tissue liver enzymes (e.g., ALT and AST from clinical chemistry)* • Other liver tissue biochemical markers (e.g., albumin; glycogen; glucose)* • Liver-specific serum biochemistry (e.g., albumin; albumin/globulin)* • Liver tissue lipids: triglycerides, cholesterol • Serum lipids	• Other liver tissue enzyme activity (e.g., catalase) or protein/DNA content are considered under mechanistic evidence for hepatic effects
Hematologic effects[b,c]	• Red blood cells* • Blood hematocrit or hemoglobin* • Corpuscular volume* • Blood platelets or reticulocytes*	• White blood cell count and globulin are under immune effects • Serum liver markers are under hepatic effects
Immune effects[b]	• Thyroid weight and histopathology • Host resistance • General immune assays (e.g., white blood cell counts, immunological factors or cytokines in blood, lymphocyte phenotyping or proliferation)* • Any measure in lymphoid tissues (weight; histopathology; cell counts; etc.) • Immune cell counts or immune-specific cytokines in non-lymphoid tissues • Other immune functional assays (e.g., natural killer cell activity, mixed lymphocyte response, phagocytosis or bacterial killing by monocytes) • Immune responses in the respiratory system	• Red blood cells are under hematologic effects • Immune responses in the respiratory tract (such as phagocytosis, cytokine signaling, inflammatory responses) are also under respiratory effects • Endpoints related to Cr(VI)-induced allergic hypersensitivity were considered under mechanistic evidence for immune effects

Relevant human health effect category	Examples of endpoints included[a]	Notes
Male Reproductive effects[b]	• Reproductive organ weight and histopathology • Markers of sexual differentiation or maturation (e.g., preputial separation) • Mating parameters (e.g., success, mount latency) • Reproductive hormones* • Sperm and semen parameters*	• Birth parameters (e.g., litter size; resorptions, implantations, viability) are under developmental effects • If data indicate altered birth parameters are likely attributable to female fertility, these data may be discussed under female reproductive effects
Female Reproductive effects[b]	• Reproductive organ weight and histopathology • Markers of sexual differentiation or maturation (e.g., vaginal opening or estrous cycling) • Birth parameters, if attributable to female fertility • Reproductive hormones*	• Birth parameters (e.g., litter size; resorptions, implantations, viability) are under developmental effects
Developmental effects[b]	• Dam health (e.g., weight gain, food consumption) • Pup viability/survival or other birth parameters (e.g., number of pups per litter) • Pup weight or growth (includes measures into adulthood after developmental-only exposure) • Developmental landmarks (eye opening, etc., but not including markers for other organ/system-specific toxicities) • Pregnancy outcomes (e.g., spontaneous abortion, early pregnancy loss, pregnancy complications, infant health, congenital malformations/anomalies) [human only]	• Histopathology and markers of development specific to other systems are organ/system-specific (e.g., vaginal opening is under female reproductive effects; offspring liver weight is under hepatic effects)
Lower respiratory effects Note: Systematic review of evidence for nasal irritation via the inhalation route will focus on data for quantitative dose-response analysis.	• Lung weight and histopathology • Biochemical markers of cell industry (e.g., total protein, albumin, and lactate dehydrogenase activity in bronchioalveolar lavage fluid) • Cellular responses (e.g., number of macrophages, neutrophils/granulocytes, and lymphocytes) • Pulmonary function (e.g., FVC, FEV1.0, DLCO) [human only]	• Immune responses in the respiratory tract (such as phagocytosis, cytokine signaling, inflammatory responses) are also under immune effects

Relevant human health effect category	Examples of endpoints included[a]	Notes
Carcinogenicity[b] Note: Systematic review of evidence for cancer via the inhalation route will focus on data for quantitative dose-response analysis.	• Tumors • Precancerous lesions (e.g., dysplasia)	

ALT = alanine aminotransferase; AST = aspartate transaminase; BMI = body mass index; DNA = deoxyribonucleic acid; LD_{50} = median lethal dose; FVC: forced vital capacity; FEV1.0: forced expiratory volume in first second; DLCO: the ratio of FEV1.0/FVC, and diffusing capacity of lung for carbon monoxide.

[a]Endpoints refer to animal data unless otherwise noted. An asterisk (*) indicates endpoints that are also measured in humans. Endpoints that are *only* measured in humans are noted by descriptive text. Some endpoints are relevant to multiple health effects. These endpoints may be categorized under only a single health effect for clarity. However, in the assessment, such outcome data may be discussed in each relevant health effect synthesis, with cross-referencing to the synthesis containing most of the evidence. The evidence (for or against an effect) will contribute to evidence integration decisions for all relevant health effects.

[b]Any of the health effect-relevant endpoints observed after developmental exposure will be discussed primarily in the health effect category indicated, and then referenced under developmental effects.

[c]The primary focus of these assessments will be on the following potential target systems: respiratory, GI tract, hepatic, hematologic, immunological, reproductive, and developmental. For cancer and nasal irritation via the inhalation route, the systematic review will focus on data that may improve the quantitative dose-response analysis, conducted in EPA's 1998 IRIS assessment, for these outcomes.

1.2.5. Dose-Response Analysis

Dose-response analysis to support derivation of toxicity values for Cr(VI) were performed consistent with EPA guidelines and support documents, especially EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b), EPA's *Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). Section 11 of the Protocol (Appendix A) describes the general approach to dose-response analysis used in this assessment.

This assessment includes development of a reference dose (RfD), a reference concentration (RfC), an inhalation unit risk (IUR), and an oral slope factor (OSF). From among the body of evidence used for the hazard identification assessment, selection of the studies for dose-response assessment used information from the study confidence evaluations, with particular emphasis on conclusions regarding the characteristics of the study population, the accuracy of the exposure estimates for epidemiology studies or dosing methods for toxicology studies, the severity of the observed effects, and the exposure levels analyzed (see Table 11-1 in U.S. EPA (2020)).

When suitable data are available, as described in Chapter 12 of U.S. EPA (2020), toxicity values should always be developed for evidence integration conclusions of **evidence demonstrates** and **evidence indicates (likely)** as well as for carcinogenicity descriptors of **carcinogenic to humans** or **likely to be carcinogenic to humans**. In general, toxicity values

would not be developed for "**evidence suggests**" for noncancer hazard or "**suggestive evidence of carcinogenic potential**" for cancer hazard conclusions, respectively.

Additional special considerations were made when selecting studies for dose-response for Cr(VI), and these are discussed in greater detail in Section 4:

- Oral animal studies which did not include an exposed group below 20 mg/kg-d were not considered for quantitative analysis[8]
- Inhalation animal studies which did not report measures of particle size and distribution were not considered for quantitative analysis[9].
- Human studies for nasal cavity effects which did not report clinical outcomes diagnosed by a trained examiner (e.g., physician, otolaryngologist, or trained researcher) were not considered for quantitative analysis. The desired clinical outcomes were atrophy of the nasal mucosa; ulceration of the nasal mucosa or septum; perforation of the septum; and bleeding nasal septum.

[8]A similar exposure consideration was not necessary for inhalation studies. Fewer animal inhalation studies were available, and concentrations were below levels that would cause severe toxicity.
[9]Availability of particle size distribution information for each study is provided in HAWC.

2. LITERATURE SEARCH AND STUDY EVALUATION RESULTS

2.1. LITERATURE SEARCH AND SCREENING RESULTS

Literature searches for studies relevant to the assessment of Cr(VI) have been conducted on a yearly basis since 2013, with the most recent update current through October 2019. This last full literature search update was conducted less than one year before the release of the draft document for public comment.

The results of the screening process outlined in Section 4.3 of the protocol (Appendix A) have been posted on the project page for this assessment in the HERO database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2233), and studies have been "tagged" with appropriate category descriptors (e.g., "included", "potentially relevant supplemental material," "excluded"). Results have also been annotated and reported in a literature flow diagram (see Figure 2-1).

Of the 13,794 unique records undergoing title and abstract screening, 10,589 were excluded because they either did not meet PECO criteria outlined in protocol Section 3.3 (Appendix A) or were screened-out using exclusion criteria outlined in protocol Section 4.3 (Appendix A). Using the sorting criteria outlined in protocol Section 4.4 (Appendix A) for studies not meeting PECO criteria but still having information relevant to the specific aims of the assessment, 3,136 records were identified. A total of 174 studies were considered eligible for study evaluation (79 human health effects studies and 85 animal health effects studies).

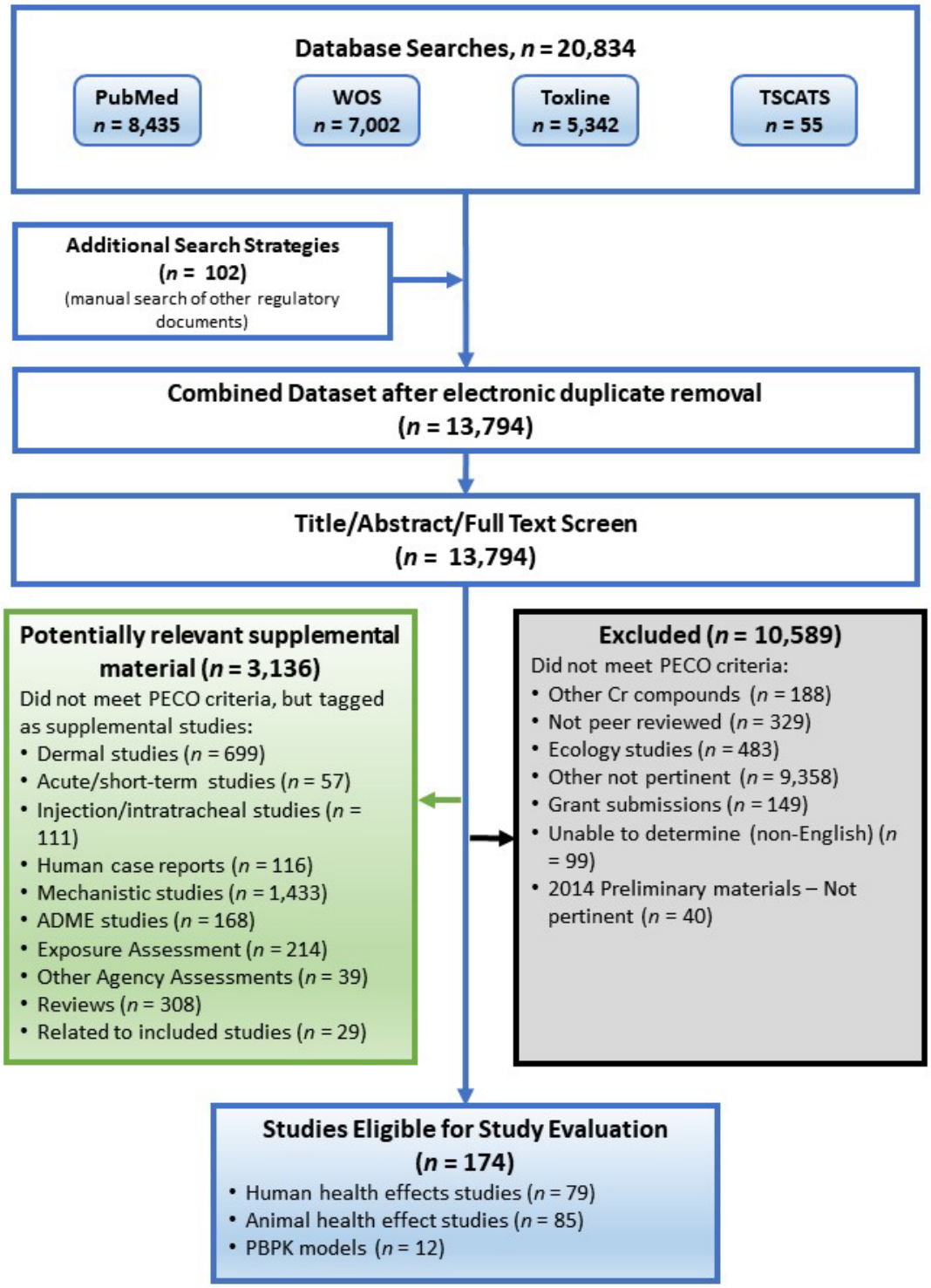


Figure 2-1. Literature search flow diagram for Cr(VI).

2.2. STUDY EVALUATION RESULTS

Human and animal studies have evaluated potential respiratory, gastrointestinal (GI) tract, hepatic, hematological, immunological, reproductive, and developmental effects following exposure to Cr(VI). The evidence informing these potential health effects is presented and assessed in Section 3.2. Detailed rationales for each domain and overall confidence rating are available in Health Assessment Workspace Collaborative (HAWC).

Overall confidence classifications are presented by effect in Section 3.2. Over 170 studies met PECO criteria (with about an even number of human and animal studies). Many human and animal studies contained information on multiple endpoints. With the exception of male reproductive effects (which had some *medium* confidence human studies), all human studies meeting PECO criteria that were included in the hazard identification analysis were rated *low* confidence for all hazard domains. Hazard domains having strong animal databases (containing *medium* and *high* confidence studies) were GI, hepatic, hematological, immune, and male and female reproductive. Most animal respiratory studies were *medium* confidence, and most of the animal developmental studies were rated *low* confidence.

For human health studies evaluated for dose-response data of nasal effects, three were considered medium (Gibb et al., 2000a; Lindberg and Hedenstierna, 1983; Cohen et al., 1974), and one was considered low (Hanslian et al., 1967). For human health studies evaluated for dose-response data of lung cancer, one was considered high (Gibb et al., 2015), one was considered medium (Proctor et al., 2016), two were considered low (Birk et al., 2006; Gerin et al., 1993), and four were considered uninformative (Girardi et al., 2015; Luippold et al., 2005; AEI, 2002; Davies et al., 1991).

Graphical representations focusing on outcome specific ratings are presented in the organ-/system-specific integration sections (Hazard Identification, Section 3.2).

3. HAZARD IDENTIFICATION

3.1. OVERVIEW OF PHARMACOKINETICS

A detailed review and literature inventory of the database regarding the absorption, distribution, metabolism, and excretion (ADME) of Cr(VI) is available in Appendix C. This section primarily focuses on Cr(VI) reduction to Cr(III) (i.e., metabolism) and localized absorption, which have the greatest impact on assessment conclusions for cancer MOA, susceptibility, interspecies differences and dose-response.

3.1.1. Pharmacokinetics

Inhaled or ingested Cr(VI) can be reduced to Cr(III) extracellularly by biological fluids (e.g., blood, gastric juices and epithelial lining fluid) of humans and rodents. In the hexavalent oxidation state, cellular uptake of chromium oxyanions occurs rapidly via ubiquitous nonspecific sulfate and/or phosphate anion transporters due to the structural similarity of the chromate and dichromate anions to these molecules (see Appendix C for more details). Once absorbed by cells, intracellular reduction generates reactive intermediates Cr(V) and Cr(IV), and finally Cr(III) (Luczak et al., 2016). In the trivalent oxidation state, chromium is poorly absorbed by cells via passive diffusion and has been shown to induce significantly lower tissue chromium burden in exposed rodents compared to Cr(VI) (Collins et al., 2010). Thus, *extracellular* reduction is believed to be a pathway for detoxification because it decreases the systemic uptake and distribution of Cr(VI) and reduces the exposure of epithelial cells, the first cells to interact with external factors, to Cr(VI). In contrast, *intracellular* reduction of Cr(VI) is considered to be a pathway for its activation following the cellular uptake of Cr(VI).

Due to site-specific Cr(VI) reduction differences by route of exposure, ingested Cr(VI) will primarily distribute to gastrointestinal (GI) tract tissues and the liver, while inhaled Cr(VI) will primarily distribute to the respiratory tract and more readily enter systemic circulation. This was demonstrated by O'Flaherty and Radike (1991), which is described in further detail in Appendix C.1.2. These pharmacokinetic factors have implications for Cr(VI)-induced toxicity and carcinogenicity because target tissue doses will strongly depend on route of exposure. An overview of ADME for inhaled and ingested Cr(VI) is provided in Figure 3-1.

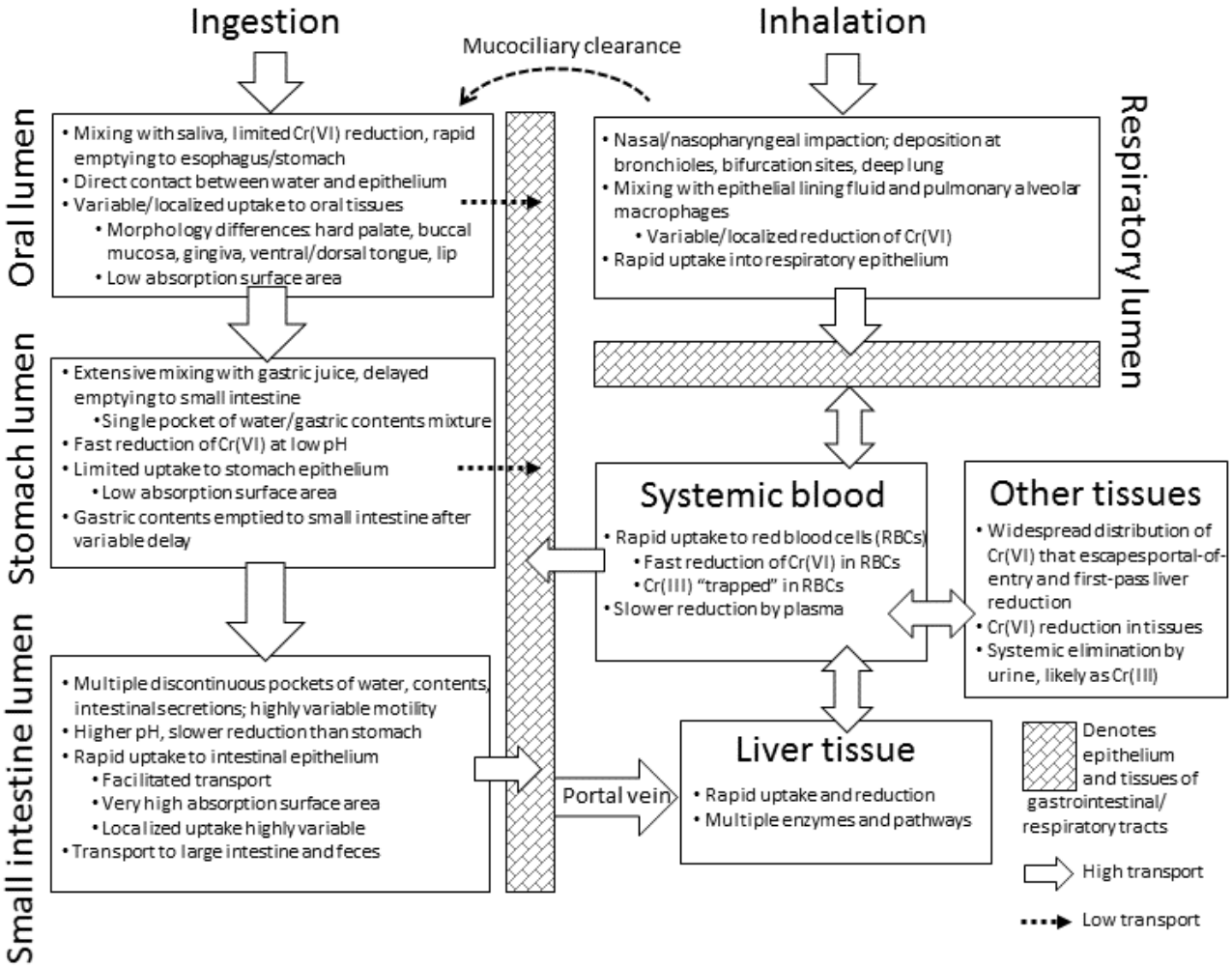


Figure 3-1. Overview of the absorption, distribution, metabolism, and excretion of Cr(VI), with focus on extracellular transport and metabolism at portals of entry.

Table 3-1 outlines the general findings regarding Cr(VI) pharmacokinetics in different organ systems, and their implications for the toxicological assessment. It is ordered from external/portal-of-entry tissues to internal/systemic tissues and provides additional support for information provided in Figure 3-1.

Table 3-1. Overall findings by system and implications for the toxicological assessment

System	General findings	Implication for assessment, with rationale
Respiratory (extracellular)	Reduction of Cr(VI) possible by epithelial lining fluid (ELF) and pulmonary alveolar macrophages (PAM). Components of lung fluids reducing Cr(VI) include glutathione (GSH) and ascorbate (Asc).[10]	**Extracellular reduction will not be quantified for inhalation dose-response modeling.** Computational fluid dynamics studies of inhaled particulates indicate that respiratory tract deposition does not occur uniformly. Thus, Cr(VI) will not evenly mix with all available reducing agent. Particulates may deposit locally in high amounts in regions of the respiratory tract with insufficient extracellular reducing capacity. Impaction in nasal/nasopharyngeal regions may also occur. Site-specific respiratory tract particle deposition and reduction may be highly variable between individuals.
Respiratory (cellular/ epithelial)	Rapid uptake of Cr(VI) into epithelial cells, and reduction to Cr(III). Reduction by lung tissue may involve peripheral lung parenchyma (PLP), Asc, GSH, cysteine, hydrogen peroxide, riboflavin, iron, and enzymatic pathways. Intracellular Cr in lung cells may cluster at the nucleus.[11]	
Oral cavity (extracellular)	Reduction in saliva is possible[12], although the extent or rate of localized reduction during the short timescale typical of human or rodent water swallowing is unknown.	**Extracellular reduction in the oral cavity will not be quantified.** Mixing of drinking water and saliva will not occur uniformly. High interindividual variability exists in oral health/saliva status and water consumption habits. Ingested water temporarily washes-away saliva from the oral cavity.
Oral cavity (cellular/ epithelial)	Uptake to the sensitive oral sites is uncertain. Higher concentrations in oral tissues were detected in mice than in rats, but only rats were susceptible to oral squamous cell carcinoma in the NTP (2008) study. Morphology within different regions of the oral cavity is highly variable (hard palate, buccal mucosa, gingiva, ventral/dorsal tongue, lip), and may impact localized uptake and reduction.[13]	**A PBPK model will not be used to estimate oral cavity absorption for dose-response modeling.** Modeling dynamics of this compartment are considered too uncertain (see above), although it will be assumed that direct contact between water and oral epithelium occurs.

[10] De Flora et al. (1987a), Petrilli et al. (1986).
[11] Wong et al. (2012), Harris et al. (2005).
[12] Petrilli and De Flora (1982).
[13] Kirman et al. (2012), Jones and Klein (2013).

System	General findings	Implication for assessment, with rationale
Stomach and intestine (extracellular/ lumen)	Gastric juices reduce Cr(VI) by 2nd-order reaction in a batch system. Total reducing capacity in all species generally between 10–30 mg/L. Components of gastric juice reducing Cr(VI) include ascorbate, glutathione, NADH, and sulfhydryls. Reduction rate decreases as pH increases.[14]	**A gastric PBPK model of the stomach will be used to estimate the Cr(VI) dose escaping stomach reduction. The adjusted daily dose may be used as the basis for an internal dose metric for dose-response modeling.** Gastric juice and Cr(VI)-containing water are expected to have time to become well-mixed, and the system is single and continuous (similar to ex vivo batch systems used to study reduction kinetics). Higher uncertainty exists for the small intestine lumen. Multiple discontinuous pockets of water/gastric contents and intestinal secretions will not be well-mixed.
Stomach and intestine (cellular/ epithelial)	Transport of Cr(VI) occurs rapidly by nonspecific phosphate and sulfate transporters. Transport of Cr(III) believed to be slower (diffusion). High variability in GI absorption for both Cr(VI) and Cr(III). Cr uptake may occur primarily in the villi. Reduction occurs in the tissue.[15]	**A PBPK model will not be used to estimate epithelial absorption of Cr(VI) in the stomach or intestine.** There is high uncertainty in simultaneously quantifying Cr(VI) uptake/reduction, and Cr(III) uptake from lumen, plasma, or background exposure. However, stomach PBPK modeling of reduction/transit is sufficient for use in dose-response modeling without incorporating uptake kinetics. In this assessment, it will be assumed that the small intestinal epithelium is exposed to any unreduced Cr(VI) escaping the stomach.
Blood	Rapid uptake of Cr(VI) into RBCs. Uptake by anion transporters (i.e., band-3 protein). Rapid reduction of Cr(VI) in RBCs by GSH. Binding to hemoglobin and other components in RBC. Transport of Cr(III) into or out of RBCs occurs slowly (thus, bound or unbound Cr(III) may be "trapped" in RBC). Cr(VI) uptake into WBCs also rapid. Reduction of Cr(VI) in plasma occurs slowly.[16]	**A systemic PBPK model will not be used to estimate whole-body pharmacokinetics.** Due to rapid clearance and reduction locally by liver, RBCs, and most other systemic tissues, $BW^{3/4}$ scaling of the available dose estimated to escape reduction in the stomach would be used for dose-response modeling for systemic endpoints outside the GI tract.
Liver	Uptake and reduction of Cr(VI) occurs rapidly. Reduction by GSH, ascorbate and other electron donors and enzymes. Uptake into cells by anion transporters.[17]	

[14] De Flora et al. (1987a), De Flora et al. (1997), Proctor et al. (2012) Kirman et al. (2013).
[15] Alexander and Aaseth (1995), Shrivastava et al. (2003), Thompson et al. (2015a).
[16] Wiegand et al. (1985), Ottenwaelder et al. (1988), Devoy et al. (2016).
[17] Alexander et al. (1982), Alexander et al. (1986), Wiegand et al. (1986), Alexander and Aaseth (1995).

System	General findings	Implication for assessment, with rationale
All other systemic organs and tissues	In vivo studies at high doses (regardless of route) have measured widespread Cr in all or most tissues examined. Distribution may be dependent on route of exposure.[18] Localized reduction of Cr(VI) to Cr(III) occurs in all tissues. Systemic elimination of Cr(III) from the whole body occurs primarily via urinary excretion. Studies also detect chromium in tissues of control animals due to background dietary or drinking water chromium (believed to be in the trivalent form).	

3.1.1.1. *Oral exposure*

The extracellular reduction process is important for the oral route of exposure due to the acidity of gastric juice that influences the reduction of Cr(VI). Cr(VI) reduction occurs more rapidly at low pH (Figure 3-2). The pH of the stomach lumen for humans and rodents in the fasted state are approximately 1.3 and 4, respectively (Figure 3-3). Under such conditions, humans would reduce Cr(VI) more effectively than rodents. Because the pH of the small intestinal lumen is higher than that of the stomach, reduction is believed to be slower once Cr(VI) is emptied from the stomach. As a result, Cr(VI) that is not reduced in the stomach compartment may traverse the remaining sections of the GI tract.

[18] O'Flaherty and Radike (1991).

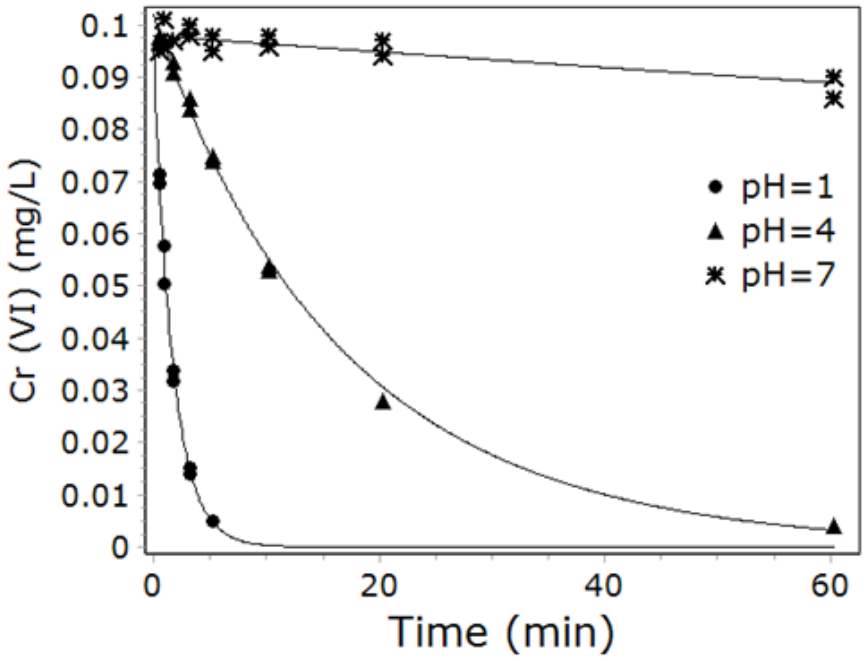


Figure 3-2. Reduction of Cr(VI) in samples of human gastric juice (fasted subjects) using data from Proctor et al. (2012). For these experiments, stomach contents were diluted 10:1 to highlight the effect of pH. Reduction of Cr(VI) in natural (undiluted) gastric juice occurs faster (see Appendix C.1.3).

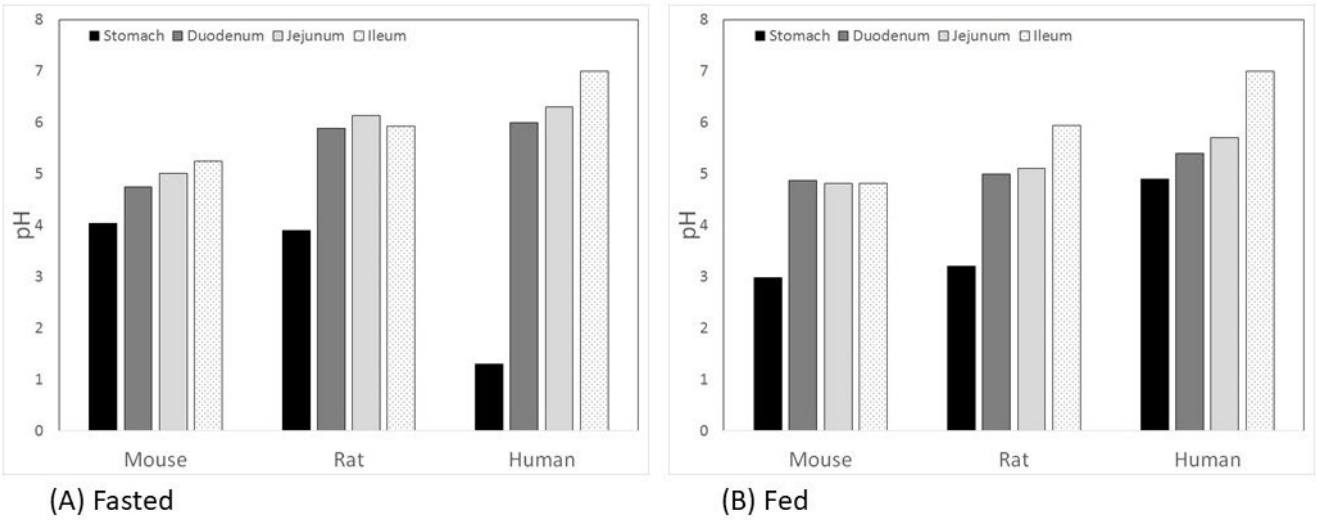


Figure 3-3. GI tract pH values reported in Mcconnell et al. (2008) (rodents: female BALB/c mice and female Wistar rats) and Parrott et al. (2009) (humans).

Along the GI tract, the concentration of Cr(VI) will be highest at the portal of entry and in the lumen proximal to the portals of entry (oral cavity, tongue, esophagus, stomach, duodenum). Within the epithelium, a concentration gradient will exist across tissue depth, with the greatest

Cr(VI) concentration at the apical surface of the mucosa, and lower levels at deeper components of the tissue. Differences in tissue morphologies and absorption across the various segments of the GI tract result in variable Cr(VI) exposures for different tissue and cell types, which have implications for site-specific uptake and pharmacodynamics (See Sections 3.2.2.3 and 3.2.3.3). Figures 3-4 and 3-5 illustrate how Cr(VI) will distribute and absorb within the GI tract tissues.

The oral epithelium is composed of multiple cell layers (Figure 3-4) (Squier and Kremer, 2001) and regenerates with stem cells located in the relatively deeper layers (e.g., the lamina propria or basal layer) (Jones and Klein, 2013; Marynka-Kalmani et al., 2010). The precise location of the stem cells depends on the region of the oral mucosa (e.g., lip, hard palate, gingiva, tongue) (Jones and Klein, 2013; Marynka-Kalmani et al., 2010). The concentration of ingested Cr(VI) in the oral cavity may not exhibit a proximal-to-distal gradient because very limited reduction and dilution will occur in the lumen. However, the surface cell layers will receive higher exposure. The small intestine is comprised of three anatomical sections, the duodenum, jejunum, and ileum (Figure 3-5), each of which have different lengths and absorption surface areas (Casteleyn et al., 2010). Within the small intestine, the concentration of ingested Cr(VI) that is not reduced in the stomach will be the highest in the duodenum. The duodenal villi serve as the functional structures for absorption. Villous epithelial cells are continuously lost and replaced by stem cells in the bottom two-thirds of the crypt (Potten et al., 2009; Potten et al., 1997). Stem cells differentiate as they move upward from the crypt and are shed at the tip of the villi. Within the stomach, gastric stem cells are located within glandular pits, and unlike the small intestine, they are nearer to the lumen and more likely to be exposed to surface irritants (Mills and Shivdasani, 2011).

There are species differences in GI tract structure and drinking water consumption patterns that may impact susceptibility to the effects of ingested Cr(VI). The rodent stomach is segmented into a glandular stomach and non-glandular (keratinized) forestomach, whereas humans have a single glandular stomach type (Kararli, 1995)[19]. Elevated pH has been measured in the forestomach of rodents (relative to the glandular stomach) (Kohl et al., 2013; Browning et al., 1983; Kunstyr et al., 1976), and pH variation might not follow the same fed/fasted pattern as the glandular stomach (Ward and Coates, 1987). As a result, it is likely that kinetics within the stomach, and Cr(VI) exposure to the absorptive regions of the stomach, differ between rodents and humans. Within the oral cavity, the location and type of tissue keratinization (which decreases site-specific absorption) differs by species, with a greater percentage of the rodent oral epithelium being keratinized relative to humans (Jones and Klein, 2013). There are also interspecies differences in the relative lengths and surface areas of small intestinal segments (Casteleyn et al., 2010). With respect to the pattern of drinking water consumption, humans ingest beverages sporadically and within a short period of

[19]A comparative 21-day pharmacokinetic study in guinea pigs (which do not have a forestomach), rats, and mice by NTP (2007) found no fundamental differences in pharmacokinetics that could be attributable to different stomach structure.

time, whereas rodents consume water at a more sustained rate over the nocturnal period (Yuan, 1993; Spiteri, 1982).

The characterization of interspecies differences in site-specific pharmacodynamics for Cr(VI) is highly uncertain due to the nature of the observed tumors (see Section 3.2.3). NTP (2008) observed tumors of the oral cavity in rats, and tumors of the small intestine of mice following exposure to Cr(VI) in drinking water for two years. The lack of oral tumors in mice cannot be explained by interspecies differences in pharmacokinetics because higher chromium concentrations have been measured in the oral tissues of mice vs. rats following a 90-day Cr(VI) drinking water study (Kirman et al., 2012). In addition, rats are generally more prone to oral cancer development than mice, and mice are more prone to neoplasia in the small intestine (Ibrahim et al., 2021; Chandra et al., 2010) (Appendix D.5).

In GI tract tissues where tumors were not observed in rodents by NTP (2008) (such as the stomach or colon), there are also interspecies differences that are difficult to model. For example, chemically-induced epithelial tumors of the forestomach in mice and rats are the most common neoplasms of the GI tract observed by NTP and Carcinogenic Potency databases, but those of the glandular stomach are rare (Chandra et al., 2010). However, glandular stomach cancer is one of the major causes of cancer diagnosis and cancer death in humans worldwide (Crew and Neugut, 2004). It is the 5th most commonly diagnosed cancer and the 7th most prevalent in the world (Rawla and Barsouk, 2019). Morphologies of stomach tumors differ greatly between humans and rodents (Hayakawa et al., 2013; Tsukamoto et al., 2007), and therefore lack of Cr(VI)-induced stomach tumors in rodent bioassays may not be directly applicable to humans. Because these interspecies differences could not be quantified in a pharmacokinetic or pharmacodynamic model, site-specific internal dose metrics were not derived for GI tract tissues.

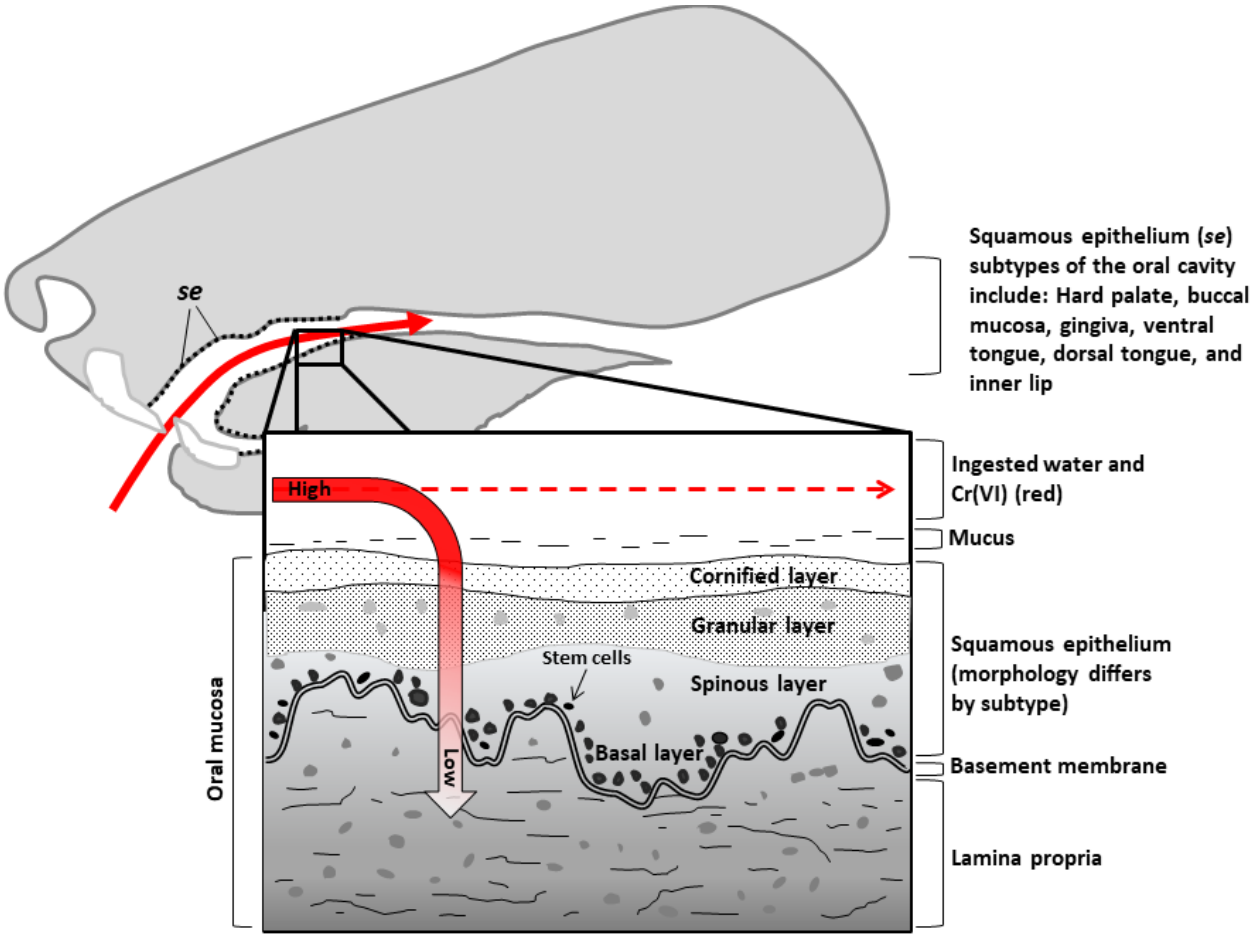


Figure 3-4. Schematic of the rat oral cavity depicting the gradient of Cr(VI) concentration following ingestion of Cr(VI) in drinking water, both from anterior to posterior locations, as well as across the tissue depth. Drawn based in part on images by NRC (2011) and Jones and Klein (2013). Transmucosal uptake may lead to systemic absorption.

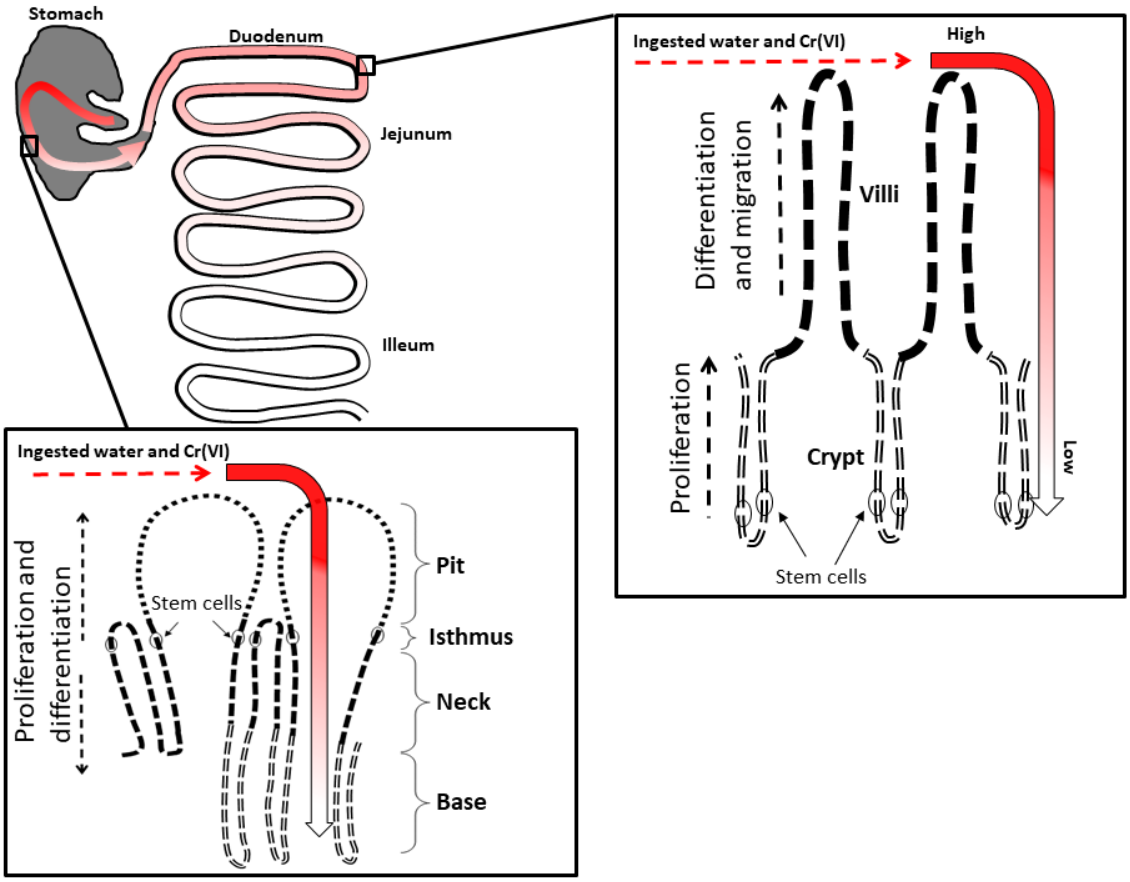


Figure 3-5. Schematic of the mouse upper GI tract (stomach and small intestine) depicting the gradient of Cr(VI) concentration following ingestion of Cr(VI) in drinking water, both from anterior to posterior locations, as well as across the tissue depth. Drawn based on images by **Radtke and Clevers (2005)**, **Fox and Wang (2007)**, and **Kararli (1995)**.

Data limitations of oral pharmacokinetic data

Even under controlled rodent pharmacokinetic studies, assessing the oral absorption and whole-body distribution of orally administered Cr(VI) at low doses contains some uncertainty. Only total chromium can be measured in tissues in vivo. Total chromium measured in tissues following oral Cr(VI) exposure results from:

1) Rapid cellular uptake of administered Cr(VI) that was absorbed into the body as Cr(VI). Because Cr(VI) transport is carrier-mediated via nonspecific sulfate and/or phosphate anion transporters, this uptake is rapid in the lumen and systemic tissues. The absorbed Cr(VI) may be transported throughout the body and reduced intracellularly to Cr(III) in tissues and red blood cells. Absorption of Cr(VI) by the intestine and reduction of Cr(VI) in the lumen are competitive processes.

1) Slow cellular uptake of Cr(III) that was absorbed into the body as Cr(III), formed from administered Cr(VI) that reduced to Cr(III) extracellularly and outside of systemic circulation (e.g., gastric juices). This process is slow and inefficient because Cr(III) transport occurs by passive diffusion, resulting in a low percent absorption of Cr(III) in the GI tract, and a low percent absorption of Cr(III) into systemic tissues from plasma. However, high concentrations of Cr(III) in the lumen may occur during controlled Cr(VI) studies (via extracellular reduction), leading to more uptake of Cr(III) than would typically occur from background dietary ingestion.
2) Slow cellular uptake of Cr(III) that was absorbed into the body as administered Cr(VI) and reduced by other components within systemic circulation (e.g., plasma, liver, red blood cells). While uptake of Cr(VI) into the intestinal lumen is rapid, systemic reduction to Cr(III) is also rapid. Once reduced, Cr(III) will diffuse slowly (into or out of) systemic tissues and circulate throughout the body in plasma. For example, plasma can reduce Cr(VI) extracellularly, and the resulting Cr(III) absorbed into tissues. RBCs can reduce Cr(VI) intracellularly, and the resulting Cr(III) can be released to systemic circulation (to be absorbed by other tissues) after RBCs are broken down.
3) Background uptake and distribution of dietary and drinking water chromium (Cr(III) and/or Cr(VI)) not administered or controlled in the bioassay. This is supported by the detection of chromium in the tissues of control animals.

Because chromium becomes trapped within RBCs following exposure to Cr(VI), elevated RBC chromium persists longer relative to plasma chromium levels following systemic Cr(VI) absorption. Based on analyses of the RBC:plasma ratios of exposed and unexposed rodents from the NTP (2008, 2007) studies (see Appendix C.1.2), it may be assumed that a significantly large percentage of oral *ad libitum* doses greater than 1 mg/kg-d likely escapes gastric and hepatic reduction in rodents and is widely distributed throughout the body. At lower doses, it may be difficult to interpret pharmacokinetic data due to background chromium exposure, and the fact that a lower percentage of the dose reaches systemic circulation.

3.1.1.2. *Inhalation exposure*

Inhalation pharmacokinetics of Cr(VI) differ substantially from ingestion, and there is less detoxification via extracellular reduction. Deposition of particles along the respiratory tract is not uniformly distributed and is strongly dependent on particle size. Inhaled particles with a diameter greater than 5 μm will typically deposit proximal to the trachea (extrathoracic region). Particles with a diameter in the range of 2.5−5 μm generally deposit in the tracheobronchial region. Particles with a diameter less than 2.5 μm generally deposit in the pulmonary region. However, some proportion of larger particles (>2.5 μm) are still capable of reaching the pulmonary region (OSHA, 2006). Deposition of both larger particles and ultrafine particles (>0.1 μm) can occur in the head airways, including the nasal passages (Hinds, 1999; ICRP, 1994). Particle size distributions in the

air vary between industries or between different processes within the same industrial plant (OSHA, 2006). Particles of respirable size capable of depositing in the lower respiratory tract have been observed in some workplace settings (Kuo et al., 1997a). As a result, this assessment assumes deposition in all regions of the respiratory tract is possible, and that some inconsistencies in observed effects may be due to particle size. Deposition and transmucosal uptake in the oral cavity are also considered to occur because humans may breathe through both the mouth and nose (Figure 3-6), as compared to nose-only breathing in rodents.

Within the lower respiratory tract of the lung, particles may locally accumulate at high quantities in susceptible areas such as airway bifurcation sites (Balashazy et al., 2003; Schlesinger and Lippmann, 1978). This is supported by studies showing high chromium deposition at these sites in the lungs of chromate workers, and a correlation between lung chromium burden and lung cancer (Kondo et al., 2003; Ishikawa et al., 1994a, b).

The respiratory environment is less acidic than the gastric environment (Krawic et al., 2017) and would be less likely to effectively reduce Cr(VI) in vivo. Unlike gastric juice, which exists in the stomach as a single continuous pocket, respiratory tract epithelial lining fluid is a thin, heterogeneous film (Ng et al., 2004). Inhaled Cr(VI) will not evenly mix with all the available extracellular components of the lung that are capable of reducing Cr(VI) to Cr(III). Thus, extracellular components capable of Cr(VI) reduction may be overwhelmed in local regions of the respiratory tract where high deposition occurs (Krawic et al., 2017), regardless of the total reducing capacity of components in the lung. As a result, PBPK modeling of extracellular Cr(VI) reduction in the lung was not considered for this assessment.

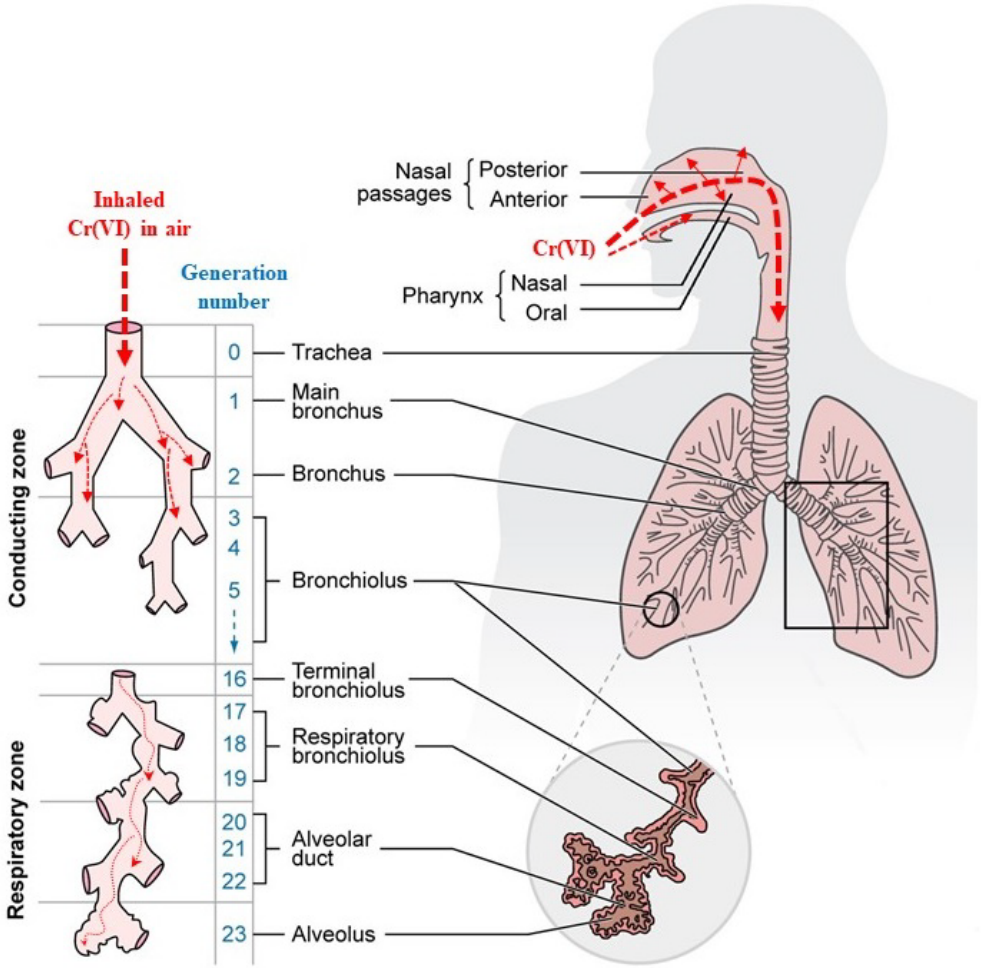


Figure 3-6. Schematics of the human respiratory system (adapted from Kleinstreuer et al. (2008)[20]) depicting deposition of particles or mists containing Cr(VI). The term generation refers to the branching pattern of airways. Each division into a major daughter (larger in diameter) and minor daughter airway is termed a generation (U.S. EPA, 1994).

Inhalation pharmacokinetics and target internal doses to the lung and systemic organs will also vary depending on the solubility of the Cr(VI) compound being inhaled. Both high and low soluble forms of Cr(VI) are believed to be absorbed into lung tissue after deposition in the airways (OSHA, 2006). However, the accumulation rates in the lung, and the extent of systemic absorption will differ. Highly soluble Cr(VI) may be rapidly absorbed by cells, leading to high localized Cr(VI) concentrations in the lung tissue. Because the highly soluble Cr(VI) would be rapidly absorbed and cleared, the high localized Cr(VI) lung concentrations may be temporary (O'Flaherty and Radike, 1991). Cr(VI) absorbed by the lungs is rapidly transported to the bloodstream and may expose

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other systemic tissues (OSHA, 2006). Cr(VI) compounds with low solubility may persist in the lung for longer periods of time and come into close contact with the bronchoalveolar epithelial cell surface (OSHA, 2006). So while uptake would be slower, there may be a higher exposure over time. Cr(VI) that is not readily absorbed into the lung may be transported to the stomach by mucociliary clearance (O'Flaherty and Radike, 1991). As a result, inhaled Cr(VI) compounds with low solubility may not reach other systemic tissues as readily as soluble Cr(VI), since most Cr(VI) swallowed by mucociliary clearance would be reduced in the stomach.

Chromium-containing compounds such as the potassium/sodium/ammonium chromates and dichromates, and chromium trioxide, are highly soluble in water, while some mixed salt chromate pigments (such as lead and zinc chromate) are poorly soluble (O'Flaherty and Radike, 1991). While stainless-steel welding fumes contain both high and low soluble components, the Cr(VI) component of the fume is considered highly soluble and may be distributed throughout the body (Antonini et al., 2010a; Antonini et al., 1999).

3.1.1.3. *Intracellular reduction (all routes of exposure)*

After Cr(VI) uptake by cells, Cr(III) is the ultimate product of the intracellular reduction of Cr(VI). Depending on the Cr(VI) concentration and reducing agent involved (e.g., ascorbate, or thiol-containing compounds such as glutathione and cysteine), various amounts of the unstable and reactive intermediates Cr(V) and Cr(IV) can be generated prior to reduction to Cr(III). This has implications for pharmacodynamics and mode of action (see Section 3.2.3.4). The reduction pathway via ascorbate occurs with a two-electron reduction to primarily produce Cr(IV) (Reynolds and Zhitkovich, 2007), although Cr(V) species have been detected following Cr(VI) reduction by ascorbate (Poljsak et al., 2005; Stearns et al., 1995; Stearns and Wetterhahn, 1994). When Cr(VI) is reduced via thiols such as glutathione, there are two distinct one-electron transfers producing both intermediates Cr(V) and Cr(IV) (Luczak et al., 2016; O'Brien et al., 2003). Both the one- and two-electron reduction steps are immediately followed by one-electron reductions to produce Cr(III) (Levina and Lay, 2005). Reduction by ascorbate is kinetically favorable with an estimated reduction rate 13x faster than cysteine and 61x faster than glutathione (Quievryn et al., 2003), and the reduction pathway via ascorbate accounts for 90% of metabolism in vivo (Standeven and Wetterhahn, 1992, 1991; Suzuki and Fukuda, 1990). It has been shown that in vitro studies may produce inaccurate results because standard cultured cells contain <1% of the normal in vivo ascorbate levels (Luczak et al., 2016). Without adequate ascorbate, glutathione is the major reducing agent, and the oxidative Cr(V) is the major intermediate; the additional Cr(V) also depletes glutathione, thereby increasing the abundance of Cr(V) (Luczak et al., 2016). In addition, the presence of ascorbate has been shown to stabilize the reactive intermediates generated by the glutathione pathway, leading to even more potential interaction between Cr(V) and intracellular components (Martin et al., 2006). These intracellular reduction pathways are summarized in Figure 3-7; for further discussion of the biological consequences of the intracellular reduction of Cr(VI), see Section 3.2.3.4.

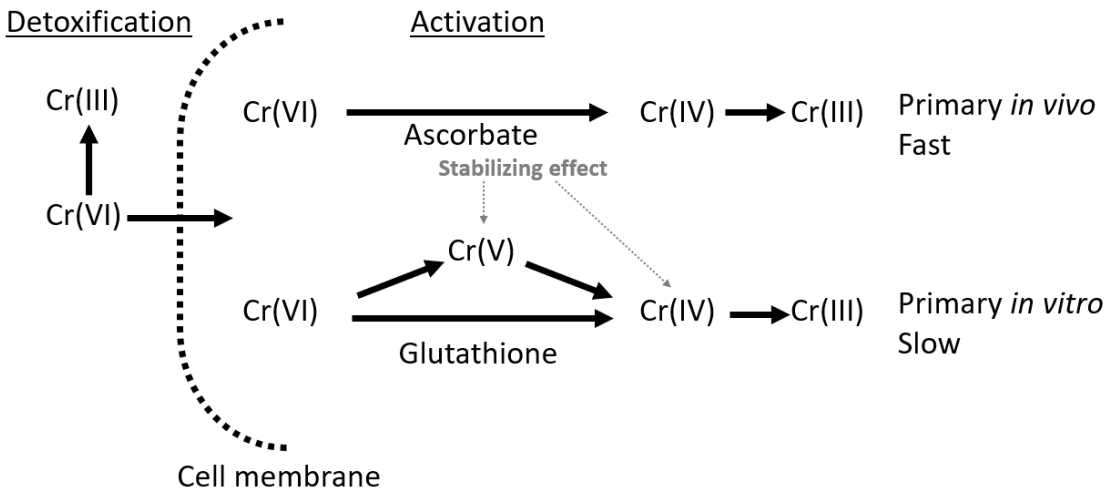

Figure 3-7. Intracellular reduction pathways of Cr(VI). Adapted from Zhitkovich (2011). The reduction pathway via ascorbate occurs with a two-electron reduction to Cr(IV), immediately followed by a one-electron reduction to Cr(III). When Cr(VI) is reduced via thiols such as glutathione, there are two distinct one-electron transfers producing the intermediates Cr(V) and Cr(IV), and lastly another electron transfer producing Cr(III). There may be uncertainty whether the ascorbate pathway truly lacks a Cr(V) intermediate (**Poljsak et al., 2005**; **Stearns et al., 1995**; **Stearns and Wetterhahn, 1994**). In vivo and in vitro differences may arise from the media and ascorbate levels used for experiments in cultured cells. Ascorbate may have a stabilizing effect on the reactive intermediates produced via the glutathione pathway.

3.1.2. Description of Pharmacokinetic Models

A brief description of the available pharmacokinetic models for Cr(VI) are listed below in chronological order in Table 3-2. For this assessment, models adapted from Sasso and Schlosser (2015); Schlosser and Sasso (2014) were used for oral dose-response and rodent-to-human extrapolation (see Appendix C). Physiology parameters defined in Sasso and Schlosser (2015) were revised to account for the fed and fasted states in humans, and to use alternative gastric physiological parameters obtained from literature and other gastric modeling platforms. A minor structural change was also made to harmonize the volumes of stomach lumen and gastric juice (see Appendix C).

Table 3-2. Pharmacokinetic models for Cr(VI)

Reference	Species	Notes
O'Flaherty (1996) O'Flaherty (1993) O'Flaherty et al. (2001) O'Flaherty and Radike (1991)	Rat	Compartments include kidney, liver, bone, GI tract, two lung pools (for inhalation only), plasma, red blood cells, and lumped compartments for remaining tissues (rapidly and slowly perfused). A single lumped compartment represents the GI tract, and reduction kinetics do not include pH-reduction relationships. This model is not readily extendable to the mouse. Calibrated to data from exposure via intravenous injection, gavage, inhalation (intratracheal), and drinking water (all data are from studies dated 1985 and earlier). Background Cr(III) exposure is simulated in the model and contributes to predicted total chromium concentrations.
O'Flaherty et al. (2001)	Human	
Kirman et al. (2012)	Rat, mouse	Compartments include kidney, liver, bone, GI tract, plasma, red blood cells and a lumped compartment for remaining tissues. A multicompartment model represents the GI tract (oral cavity, stomach, duodenum, jejunum, ileum, large intestine), with reduction kinetics based on the model by Proctor et al. (2012). Incorporates pharmacokinetic data from experiments designed by the study authors, and data from other studies. Only data for drinking water and dietary routes of exposure incorporated. Total concentrations in control groups subtracted from exposure groups to account for background Cr(III) exposure.
Kirman et al. (2013)	Human	
Schlosser and Sasso (2014); Sasso and Schlosser (2015)	Rat, mouse, human	Simulates Cr(VI) reduction kinetics and transit in the stomach. Incorporates pharmacokinetic model of the stomach lumen by Kirman et al. (2013; 2012), but with a revised model for Cr(VI) reduction based on reanalysis of ex vivo data to improve model/data fit.
Kirman et al. (2017; 2016)	Rat, mouse human	Same structure as Kirman et al. (2013; 2012), but incorporates a revised model for Cr(VI) reduction based on additional human gastric juice data. This model supersedes earlier models by the same investigators.
ICRP (Hiller and Leggett, 2020)	Human	Biokinetic model assuming linear 1st-order transfer rates among different systemic tissues. Compartments include respiratory tract, stomach, small intestine, red blood cells, plasma, liver, kidneys, other/soft tissue, trabecular bone, cortical bone, right colon, left colon, rectosigmoid colon, urinary bladder, urine, feces. Reduction of Cr(VI) to Cr(III) not explicitly modeled (assumed as a linear transfer between different special plasma compartments).

The O'Flaherty Cr(VI) model was adapted from a PBPK model for lead, and it does not describe Cr(VI) kinetics in the target tissue or species of concern (the mouse GI tract). The models by Kirman et al. (2013; 2012) simulate interspecies differences in gastric reduction kinetics in mice, rats, and humans. These models have a structure similar to the human model by O'Flaherty et al. (2001), but differ in their simulation of background Cr(III) exposure and kinetics of the GI tract and bone. The model presented in Sasso and Schlosser (2015) and Appendix C.1.5 only incorporates the

GI lumen compartments necessary to simulate the non-systemic dose metrics. It incorporates in vivo gastric kinetics from the Kirman et al. (2013; 2012) models, but includes a revised ex vivo reduction model by Schlosser and Sasso (2014) to improve model fit to the ex vivo data of Proctor et al. (2012) and Kirman et al. (2013). Models of the GI tract incorporate ex vivo reduction models and may be run independently of the rest of the body if the internal dose is not impacted by blood or tissue concentrations (Figure 3-8). Some internal dose metrics for GI tract toxicity do not require estimates of tissue absorption, blood concentrations or systemic elimination. Validation of whole-body pharmacokinetics is complicated by background exposure and inability to speciate chromium oxidation states in vivo (see Section ES.7 and 3.1.1.1).

The ICRP model (Hiller and Leggett, 2020) was focused heavily on the distribution of Cr(III) in the body and had an over-simplified linear assumption for Cr(VI) reduction that would be inadequate for assessment of effects in the GI tract.

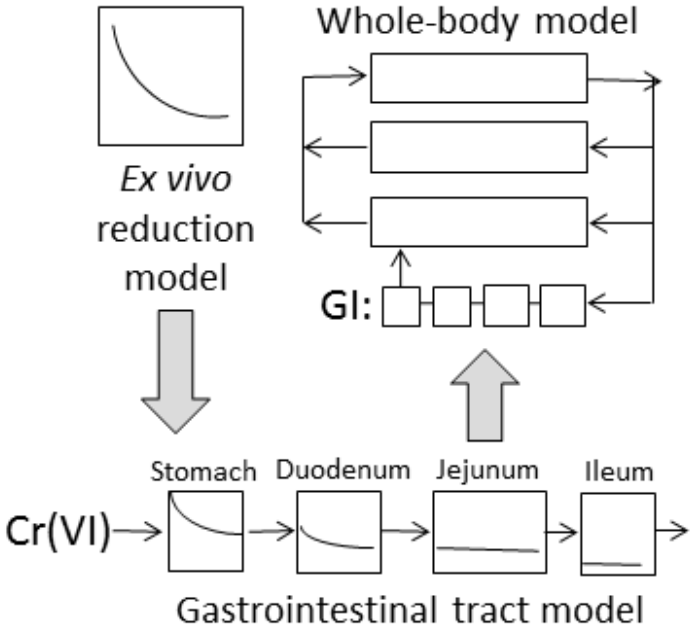

Figure 3-8. Relationship between ex vivo reduction models, in vivo gastric models, and whole-body PBPK models.

The Kirman et al. (2017) model made revisions to the previous Kirman et al. models by incorporating some ex vivo reduction concepts presented the Schlosser and Sasso (2014) (such as multiple-pathway reactions), and is calibrated to human gastric juice data for fed and fasted individuals (Kirman et al., 2016). Ex vivo data provided in Kirman et al. (2016) and De Flora et al. (2016) were used to assess model uncertainties and population variability and develop a fed-state gastric reduction capacity (see Appendix C.1). Minor updates to the Sasso and Schlosser (2015) in vivo model structure and physiology are documented in Appendix C.1.5.

3.1.2.1. *Rationale for using a gastric PBPK model*

This toxicological review applies models describing the reduction kinetics and transit of Cr(VI) in the stomach lumen (as opposed to whole-body PBPK models) for the oral dose-response assessment and rodent-to-human extrapolation (Appendix C.1.5).

In the GI tract, the extent of reduction in the stomach compartment determines the maximum Cr(VI) mass or concentration that enters the small intestine. As a result, the stomach compartment is a major contributor to inter- and intraspecies pharmacokinetic variation. If reduction does not occur effectively in the stomach, a greater amount of unreduced Cr(VI) will persist in the small intestinal compartments (duodenum, jejunum, and ileum). Since values of pH in the small intestinal compartments are higher than in the stomach for all species (Figure 3-3), reduction may occur less effectively once chromium has emptied from the stomach. Furthermore, the data underlying the ex vivo reduction model were generated under batch reaction conditions, which is more similar to the stomach compartment than the dynamic intestine. Modeling the stomach requires less extrapolation of the data.

The gastric PBPK models are consistent with both ex vivo and in vivo pharmacokinetics studies. It is estimated that approximately 10% of an ingested dose of Cr(VI) is absorbed in the GI tract of rodents (Fébel et al., 2001; Thomann et al., 1994), and this is consistent with the percentage of unreduced Cr(VI) emptying from the stomach predicted by the gastric PBPK model (Appendix C). Under typical physiological conditions in the human (gastric pH of below 3, and gastric emptying half-time of approximately 15–30 minutes), gastric PBPK models predict that approximately 1–10% of ingested Cr(VI) may be emptied by the human stomach unreduced. This is in agreement with pooled human gastric juice data by De Flora et al. (2016), which showed that approximately 93% of the chromium is reduced by undiluted gastric juice after 15 minutes. This is also consistent with a Cr(VI) bioavailability study performed in an in *vitro system*, which found that human bioaccessibility could be as high as 20% at low doses (0.005 mg/kg-d) at a gastric pH of 3.0 (but drastically lower than 20% at low pH)(Wang et al., In Press) (in press). Elevated chromium biomarkers (plasma, red blood cells and urine) have been measured in human volunteers ingesting Cr(VI) (Finley et al., 1997; Kerger et al., 1997; Kerger et al., 1996; Paustenbach et al., 1996).

While reduction may still occur in small intestinal compartments, effects observed by NTP (2008) in mice (see Sections 3.2.2 and 3.2.3.2) indicate that unreduced Cr(VI) may traverse the small intestine. The jejunum and ileum exhibited lower incidences of effects in mice, which may indicate that Cr(VI) was reduced and/or diluted by intestinal secretions and lumen contents. Data by Kirman et al. (2012) also shows chromium concentrations decreasing in the distal direction in the small intestine of mice exposed to Cr(VI) in drinking water for 90 days. While it is believed that more Cr(VI) is absorbed in the proximal small intestine, this assessment will not quantify spatial differences in absorption within the small intestine. It will be assumed that all Cr(VI) which escapes the stomach and enters the small intestine is capable of exposing the intestinal epithelium of any region.

3.2. SYNTHESIS AND INTEGRATION OF HEALTH HAZARD EVIDENCE BY ORGAN/SYSTEM

3.2.1. Respiratory Tract Effects Other Than Cancer

The respiratory tract is comprised of multiple tissues that are responsible for air intake and gas exchange. The upper respiratory tract is composed of the nose, nasal cavity, mouth, pharynx and larynx. This region filters, warms and humidifies inhaled air prior to entering the lower respiratory tract, while also facilitating olfactory function. The lower respiratory tract (i.e., tracheobronchial and pulmonary regions), which begins at the larynx below the vocal cords, is composed of the trachea, bronchi, bronchioles, and the alveoli. The pulmonary region facilitates gas exchange with the blood. The upper and lower airways and gas-exchange region can be affected by inhaled toxicants that are deposited along the different regions of the respiratory tract, resulting in a variety of adverse respiratory outcomes. For an overview of how the particle size and solubility of Cr(VI) compounds will impact the retention and absorption of Cr(VI) in different regions of the respiratory tract, see Section 3.1.

Effects in the nasal cavity (irritation/ulceration of the nasal mucosa or septum, perforation of the septum, and bleeding nasal septum) have been documented for decades in humans occupationally exposed to Cr(VI) in chromium-related industries (Bloomfield and Blum, 1928). As stated in the Cr(VI) IRIS Assessment Protocol (Appendix A), based on EPA's 1998 evaluation of the literature and the determination that the effects of Cr(VI) on the nasal cavity have been well established [e.g., OSHA (2006) and U.S. EPA (2014c)], EPA will not re-evaluate the qualitative evidence for an association between inhalation Cr(VI) exposure and nasal effects. Rather, the review of the evidence for nasal effects focuses on identifying studies that might improve the quantitative dose-response analysis for this outcome. The review of the evidence and dose-response for nasal effects can be found in Section 3.2.1.

For human studies, this assessment focuses on respiratory effects that may be sensitive and specific to the effects of inhaled Cr(VI) exposure. This includes decrements in lung function assessed using spirometry, with comparisons against lesser or unexposed individuals. Mortality or self-reported symptoms (such as cough) that are nonspecific and may be attributed to multiple other causes were not considered relevant for this assessment. For animal bioassays, this assessment considered relevant any reported respiratory effects. Animal studies of respiratory effects following Cr(VI) exposures typically focused on cellular responses (i.e., cell recruitment, cell function and cellular products), histopathology, and lung weight.

3.2.1.1. *Human Evidence*

Study evaluation summary

Table 3-3 summarizes the human studies considered in the evaluation of the effects of exposure to Cr(VI) on the lower respiratory tract. These comprise four occupational cohort studies

of workers in industrial settings in which exposure to Cr(VI) is known to occur (predominantly through inhalation): a chrome electroplating department in Taiwan (Kuo et al., 1997b), a chromate production plant in China (Li et al., 2015b), a chrome electroplating plant in Sweden (Lindberg and Hedenstierna, 1983), and several plants in France at which stainless-steel welding was performed (Sobaszek et al., 1998). Five additional studies were considered but were deemed *uninformative* due to critical deficiencies (Sitalakshmi et al., 2016; Sharma et al., 2012; Huvinen et al., 2002b; Nielsen et al., 1993; Bovet et al., 1977) and are not further discussed (see HAWC for additional details).

Concentrations of Cr(VI) in air were measured in three of the four studies. Concentrations of Cr(VI) from stationary monitors and personal samplers at a chrome-plating facility in Sweden ranged from <0.2 to 46 μg/m^3 (Lindberg and Hedenstierna, 1983). Concentrations of Cr(VI) from personal samplers ranged from 0.2 to 230.0 μg/m^3 in a study of chromium electroplaters in Taiwan (mean [SD]: 63.2 [67.2] μg/m^3 (Kuo et al., 1997a, b)). Air concentrations from stationary monitors were slightly lower (median [quartile][21]: 15.45 [19] μg/m^3) in the study of chromate workers in China (Li et al., 2015b). The use of cellulose fiber filters instead of PVC filters as recommended by NIOSH may have resulted in underestimated air concentrations in the latter study (see HAWC for additional details).

After study evaluation, all four studies were categorized as *low* confidence (Li et al., 2015b; Sobaszek et al., 1998; Kuo et al., 1997b; Lindberg and Hedenstierna, 1983). A lack of air or biomarker measurements in the study of stainless-steel welders (Sobaszek et al., 1998), and potential for residual confounding in the other studies (Li et al., 2015b; Kuo et al., 1997b; Lindberg and Hedenstierna, 1983), raised concerns about the ability of these studies to appropriately characterize respiratory effects and resulted in *low* confidence ratings despite other notable strengths in terms of study design and methods. In all the considered studies, while the primary focus was on chromium exposure, co-exposure to other occupational hazards may also contribute to observed health effects. For example, other metallic elements in welding fumes or nickel in electroplating work, could also impact respiratory health (Antonini et al., 2010b; ATSDR, 2005). However, similar effects on respiratory outcomes from studies conducted across different occupational settings, where the specific co-exposures would be expected to differ, would alleviate concern that any observed effects are due solely to co-exposures rather than to Cr(VI).

The main results of the four studies considered are summarized in Table 3-4.

[21]The article states this value as median and quartile; this appears consistent with an inter-quartile range.

Table 3-3. Summary of human studies for Cr(VI) lower respiratory effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Study Design	Pulmonary Function
Kuo et al. (1997b) (related: Kuo et al. (1997a))	Chrome electroplating	Taiwan	Cohort (occupational)	L
Li et al. (2015b)	Chromate production	China	Cohort (occupational)	L
Lindberg and Hedenstierna (1983)	Chrome electroplating	Sweden	Cohort (occupational)	L
Sobaszek et al. (1998)	Stainless-steel welding	France	Cohort (occupational)	L

[a]Studies excluded due to critical deficiency in one or more domains: Nielsen et al. (1993), Bovet et al. (1977), Sharma et al. (2012), Sitalakshmi et al. (2016), and Huvinen et al. (2002b) (related: Huvinen et al. (1996)). One of these studies (Bovet et al., 1977) met the PECO criteria but was found to be uninformative at the study evaluation stage due to publication prior to the availability of standardized spirometry guidelines from the American Thoracic Society.

Synthesis of human evidence

Pulmonary function

Four core endpoints were considered in the evaluation of the effects of exposure to Cr(VI) on pulmonary function: forced vital capacity (FVC), forced expiratory volume in first second (FEV1.0), the ratio of FEV1.0/FVC, and diffusing capacity of lung for carbon monoxide (DLCO). The first three of these are measured by spirometry. Other tests of pulmonary function (such as peak flow, airway responsiveness, and lung volume) were not utilized in any of the four studies considered. A key consideration for the evaluation of spirometry data is the adherence to guidelines published by the American Thoracic Society (ATS) (ATS/ERS, 2019)[22] and use of appropriate reference population data for estimation of predicted values. The results from the four studies evaluating spirometry endpoints are shown in Tables 3-4 and 3-5.

[22]These guidelines first developed in 1979 with subsequent updates; standardized guidelines were harmonized with the European Respiratory Society beginning in 2005 with subsequent updates and include detailed standardized protocols for the collection of spirometry data. Key features of the ATS guidelines include: recommendations regarding spirometer equipment specifications; protocols to be followed during the administration of spirometry tests; and the importance of considering age, sex, and height when interpreting results (ideally by expressing spirometry measurements as a percent of the measurement predicted, using reference values appropriately matched to the demographic characteristics of the study population).

Table 3-4. Summary of results from human studies of effects of Cr(VI) exposure on pulmonary function

Study	Exposure	Conf.	Result Format	N	FVC	FEV	FEV/FVC
Li et al. (2015b)	Chromate production Median total Cr[a] measured in air: 15.45 µg/m^3 (exposed) and 0.23 (referent) µg/m^3	L	Mean (SD) expressed as a percent of predicted values.	Exp: 91 Ref: 38	Exp: 72.34 (14.18) Ref: 81.01 (20.79) p = 0.196	Exp: 76.04 (16.20) Ref: 86.71 (24.53) p = 0.011	Exp: 116.18 (11.32) Ref: 114.08 (10.79) p = 0.044
Kuo et al. (1997b)	Chrome electroplating Mean Cr(VI) measured in air near electroplating tank: 8.0 µg/m^3 (Cr factors), 2.8 µg/m^3 (Cr-Ni factory) and <LOD (Zn factory) (published separately in Kuo et al. (1997a); unclear whether for the same factories included in the study)	L	Adjusted regression coefficients (SE) and p-value	Exp: 26 Ref: 34	β: −556.4 (151.2) mL p < 0.01	β: −368.0 (163.9) mL p < 0.05	-
Lindberg and Hedenstierna (1983)	Chrome electroplating Cr(VI) exposure categories were low (<2 µg/m^3), high (≥2 µg/m^3) or mixed exposure to chromic acid and other acids and metallic salts	L	See table below	Multiple comparison groups. See table below	See table below	See table below	-
Sobaszek et al. (1998)	Stainless-steel welding No quantitative exposure measures	L	Mean (SD) expressed as a percent of predicted values.	Exp: 130 Ref: 234	Exp: 103 (12) Ref: 101 (13) NS	Exp: 99 (15) Ref: 98 (14) NS	Exp: 95 (8) Ref: 96 (8) NS

[a]Total Cr includes Cr(III) and Cr(VI). No quantitative Cr(VI) exposure measurements reported.

Table 3-5. Summary of results from Lindberg and Hedenstierna (1983) study of effects of Cr(VI) exposure on pulmonary function

Study information	N	FVC	FEV
Exposure Chrome electroplating **Study confidence** Low **Result format** Mean (SD) expressed as actual volume (Liters of air) Note: Measurements were taken Monday morning before work, Thursday morning before work, and Thursday afternoon after work	Males only, Monday morning before work: Exp: 26 nonsmokers Exp: 48 smokers Ref: 52 nonsmokers Ref: 67 smokers	Nonsmokers, Exp: 5.61 (0.99) Nonsmokers, Ref: 5.20 (1.00) NS Smokers, Exp: 5.27 (0.90) Smokers, Ref: 5.66 (1.02) NS	Nonsmokers, Exp: 4.54 (0.92) Nonsmokers, Ref: 4.08 (0.85) NS Smokers, Exp: 4.31 (0.85) Smokers, Ref: 4.38 (0.92) NS
	Males and females, Non-smoker, High Exp (n = 6)	Mon. morning: 5.96 (1.64) Thurs. afternoon: 5.75 (1.58) $p < 0.01$	Mon. morning: 5.13 (1.37) Thurs. afternoon: 4.92 (1.29) $p < 0.05$
	Males and females, Non-smoker, Low Exp (n = 10)	Mon. morning: 5.41 (1.27) Thurs. afternoon: 5.35 (1.24) NS	Mon. morning: 4.45 (1.05) Thurs. afternoon: 4.43 (0.97) NS
	Males and females, Non-smoker, Mixed Exp (n = 15)	Mon. morning: 4.93 (1.17) Thurs. afternoon: 4.73 (1.22) $p < 0.01$	Mon. morning: 4.12 (0.92) Thurs. afternoon: 4.06 (0.95) NS
	Males and females, Smoker, All Exp (n = 48)	Mon. morning: 5.04 (1.04) Thurs. afternoon: 4.97 (0.97) $p < 0.05$	Mon. morning: 4.07 (0.95) Thurs. afternoon: 4.00 (0.91) NS

One *low* confidence study (Li et al., 2015b) reported lower FVC and FEV1.0 in chromate workers compared to referents (workers in the same plant in administrative offices) with little to no exposure to Cr(VI) in China (Li et al., 2015b) (Table 3-4). The percent predicted values for FVC and FEV1.0 in the exposed group were 72.34 (SD:14.18) and 76.04 (SD: 16.20), respectively, compared with 81.01 (SD: 20.79) and 86.71 (SD: 24.53), respectively, in the referent group. The low percent predicted values in both the exposed and referent groups may in part reflect the high prevalence of smoking (39.56% of exposed and 28.95% of unexposed workers were current smokers), which was not accounted for in these analyses. Another possible reason for low percent predicted values across groups is that the referent group had undescribed exposure to Cr(VI) or other respiratory toxicants. Finally, it is possible that use of reference values from an ethnically different population (in this case, Japanese and European referent populations, per correspondence with study author (Jia, 2021)) could have resulted in low percent predicted values (Korotzer et al.,

2000). The use of an inappropriate referent to estimate predicted pulmonary function measures may not impede comparisons of FVC and FEV1.0 between groups within the same study; however, the impact could differ for FVC compared with FEV1.0, thus there is greater uncertainty in FEV1.0/FVC results (mean [SD]: 116.18 [11.32] in exposed, 114.08 [10.79]). One *low* confidence study (Li et al., 2015b) reported lower FVC and FEV1.0 in chromate workers compared to referents (workers in the same plant in administrative offices) with little to no exposure to Cr(VI) in China (Li et al., 2015b) (Table 3-4). The percent predicted values for FVC and FEV1.0 in the exposed group were 72.34 (SD:14.18) and 76.04 (SD: 16.20), respectively, compared with 81.01 (SD: 20.79) and 86.71 (SD: 24.53), respectively, in the referent group. The low percent predicted values in both the exposed and referent groups may in part reflect the high prevalence of smoking (39.56% of exposed and 28.95% of unexposed workers were current smokers), which was not accounted for in these analyses. Another possible reason for low percent predicted values across groups is that the referent group had undescribed exposure to Cr(VI) or other respiratory toxicants. Finally, it is possible that use of reference values from an ethnically different population (in this case, Japanese and European referent populations, per correspondence with study author (Jia, 2021)) could have resulted in low percent predicted values (Korotzer et al., 2000). The use of an inappropriate referent to estimate predicted pulmonary function measures may not impede comparisons of FVC and FEV1.0 between groups within the same study; however, the impact could differ for FVC compared with FEV1.0, thus there is greater uncertainty in FEV1.0/FVC results (mean [SD]: 116.18 [11.32] in exposed, 114.08 [10.79]).

Another *low* confidence study comparing chrome electroplaters to zinc electroplaters in Taiwan (Kuo et al., 1997b) reported average FVC and FEV values were 556.4 mL (SD: 151.2, $p < 0.01$) and 368.0 mL (SD 163.9, $p < 0.05$) lower, respectively, in the group of chrome electroplaters after adjusting for age and sex (Table 3-4). However, height (an important predictor for these measures) was not accounted for in comparison of spirometry values.

A *low* confidence study of chromium electroplaters in Sweden (Lindberg and Hedenstierna, 1983) (Table 3-5) did not find significant differences between FVC or FEV1.0 comparing those with low and high average exposure to chromic acid, nor when comparing exposed workers and a referent group of auto mechanics. However, when evaluating spirometry measurements over the course of the work week (pre-shift on Monday morning vs. post-shift on Thursday afternoon), there were significant decrements in both measures for those in the high exposure group. This finding demonstrates the potential for short-term effects of chromic acid exposure to impact lung function within the same individual and is not affected by the potential for confounding by age and height that is a primary concern for the comparison of exposed and referent group lung function measures; however, it does not inform the difference between workers exposed to chromic acid and referent workers.

The fourth *low* confidence study (Sobaszek et al., 1998) also did not report significant differences in FVC, FEV1.0 (or the ratio of FEV1.0/FVC) between exposed and referent groups

(Table 3-4). There were no major concerns regarding selection bias, outcome measurement, or statistical analyses in this study, which presented results as a percent of predicted values and followed ATS protocols. Rather, the low confidence rating arose from concerns about the ability of the study to detect an association in the presence of exposure misclassification arising from the lack of quantitative exposure data (Sobaszek et al., 1998). However, an additional analysis conducted in this study may provide supporting evidence of an association between chronic exposure to stainless-steel welding fume and decreased pulmonary function. In this analysis, maximal expiratory flow (MEF) first increased and then decreased with exposure quantified as years of duration in welding. The initial increase in MEF may indicate that more susceptible workers quickly left the workforce (i.e., healthy worker effect). Subsequently, the remaining workers experienced a decrease in MEF after long-term exposure to stainless-steel welding fumes (more than 25 years), a pattern that is consistent with the results of the *low* confidence study reporting decreases in pulmonary function in workers exposed to Cr(VI) compared to lesser exposed workers (Li et al., 2015b).

Overall, there is an indication in two *low* confidence human studies that higher Cr(VI) exposure is associated with decrements in lung function assessed using spirometry, and the two remaining *low* confidence studies may have had insufficient sensitivity to appropriately characterize such associations.

3.2.1.2. *Animal Evidence*

Study evaluation summary

The eight animal toxicology studies that were considered in the evaluation of the effects of Cr(VI) on the respiratory tract are summarized in Table 3-6. All of these studies used the inhalation route of exposure (nose only or whole body) using respirable aerosols[23] and examined respiratory effects in male rats, mice, and rabbits. Female animals were not assessed. The exposure duration for the mouse studies was 2 years, while the rabbit studies were limited to 4–6 weeks. The rat studies ranged from 4 weeks to 18 months.

The outcomes reported can be generally grouped into three categories: cellular responses, lung histology and lung weight. Cellular responses include cell recruitment (the transfer of vascular cells; monocytes, granulocytes/neutrophils and lymphocytes into the airways), cell function (macrophage phagocytosis) and release of cellular products (proteins and enzymes). Cell recruitment is evaluated using bronchoalveolar lavage (BAL) to obtain total cell counts, and relative abundance of the various resident and recruited populations of cells recovered in the BAL fluid (BALF) including monocytes, macrophages, granulocytes/neutrophils and lymphocytes. Cell

[23]For study quality evaluation, consideration was given to reporting (or lack of reporting) of particle size and distribution (such as mass median aerodynamic diameter [MMAD] and geometric standard deviation [GSD]). Lack of reporting on particle sizes negatively impacted the *exposure methods sensitivity* rating and *overall confidence* rating.

function is evaluated by measuring the ability of macrophages to phagocytose foreign particles and their ability to release protective oxidant enzymes. Cellular products released by protective cells within the lumen of the lung that can be measured in the BALF include cytokines, intracellular enzymes and proteins, as well as other cell signaling chemicals.

The majority of the study outcomes focusing on cellular responses and histopathology were rated as medium confidence with minor concerns that did not negatively affect the overall outcome confidence rating. Five study outcomes were rated as *low* confidence (four of these were for lung weight, and one was for lung histopathology), and one was rated *uninformative* (Table 3-6).

Table 3-6. Summary of included studies for Cr(VI) respiratory effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive data graphic for rating rationales.

				Respiratory outcomes		
Author (year)	**Species (strain)[b]**	**Exposure design**	**Exposure route**	**Cellular responses**	**Histopathology**	**Lung weight**
Cohen et al. (2003)	Rat (F344)	4, 8, 12, 24, 48 wk	Inhalation	M		
Glaser et al. (1985)	Rat (Wistar)	28 and 90 d	Inhalation	M	M	M
Glaser et al. (1990)	Rat (Wistar)	30 d, 90 d, and 90 d with 30 d recovery	Inhalation	M	M	L
Kim et al. (2004)	Rat (Sprague-Dawley)	90 d	Inhalation		M	L
Johansson et al. (1986a)	Rabbit (not specified)	4–6 wk	Inhalation		M	L
Johansson et al. (1986b)	Rabbit (not specified)	4–6 wk	Inhalation	M	M	
Nettesheim et al. (1971)	Mouse (C57BL/6)	2 yr	Inhalation		L	
Glaser et al. (1986)	Rat (Wistar)	Chronic	Inhalation		U	L

[a]In addition to these studies, there was one study Nettesheim et al. (1970) that met the PECO criteria but was found to be *uninformative* at the study evaluation stage due to incomplete reporting of histopathological findings in all the groups. A group of foreign language studies (Adachi et al., (1987; 1986; 1981)) were determined to be *uninformative* (English-language abstract and results indicated that the exposure vehicle purposefully contained additional contaminants in order to simulate a chromic acid bath). Noncancer histopathology in Glaser et al. (1986) was rated uninformative due to incomplete reporting of histopathological findings in all the groups.
[b]All data are for male animals

Synthesis of animal evidence

Lung Cellular Responses in BALF

When particulate matter is inhaled, the lungs typically respond by increasing phagocytic cell populations to aid in clearance of the particles. Populations of macrophages in the lung increase by replication of the resident lung macrophages (Bitterman et al., 1984), as well as by recruitment of monocytes from the bloodstream that travel to the lung and mature to macrophages (van Oud Alblas and van Furth, 1979). In addition, granulocytes (i.e., neutrophils) can be recruited to assist in the phagocytosis of the foreign particles (Kodavanti, 2014). These changes in cell populations, indicative of inflammation, may be accompanied by biochemical markers of cell injury, such as changes in the amounts of total protein, albumin, and lactate dehydrogenase (LDH) activity in BALF (Henderson, 1984). These cellular responses are protective immediately following exposure but can become injurious to the organism if they are prolonged, leading to long-term changes such as increased alveolar-capillary permeability (pulmonary edema).

Four of the included studies reported cellular response outcomes, all of which had *medium* confidence ratings. Laboratory animals exposed to aerosols of Cr(VI) exhibited changes in the protective cells that reside in or recruit to the lung. Findings included changes in the number of macrophages, granulocytes/neutrophils, and lymphocytes, as well as changes in the total BAL cells. Chromium concentration-related changes in the number of macrophages recovered in the BALF were observed in all four studies (Cohen et al., 2003; Glaser et al., 1990; Johansson et al., 1986b; Glaser et al., 1985), although the direction of the effects were not consistent across studies or durations of exposure (Figure 3-9).

Statistically significant increases in numbers of alveolar macrophages in BALF were reported in male rabbits exposed to 0.9 mg/m^3 Cr(VI) as sodium chromate aerosol for 4–6 weeks (Johansson et al., 1986b) and in male Wistar rats exposed to Cr(VI) as sodium dichromate at concentrations of 0.20 and 0.40 mg/m^3 for 30 or 90 days (Glaser et al., 1990). In contrast, Glaser et al. (1985) reported no significant changes in the number of BALF macrophages in male Wistar rats after 28 days of Cr(VI) exposure, and a significant concentration-dependent decrease in the number of BALF macrophages from rats exposed to Cr(VI) concentrations of 0.050 and 0.20 mg/m^3 for 90 days. The numbers of BALF macrophages in F344 rats exposed to Cr(VI) in the form of calcium chromate aerosol (0.36 mg/m^3) for durations of 4, 8, 12, 24, and 48 weeks were decreased relative to controls at most intervals (Cohen et al., 2003).

While data for the number of BALF macrophages were variable in the available studies, macrophages were shown by one research group to undergo replication as a consequence of Cr(VI) exposure via inhalation. Significant increases in specific macrophage populations including polynuclear macrophages (Glaser et al. (1985), 90 day, LOAEL 0.05 mg/m^3), macrophages in telophase (Glaser et al. (1985), 90 day, LOAEL 0.025 mg/m^3) and dividing macrophages (Glaser et al. (1990), 90 day, LOAEL 0.05 mg/m^3) were observed in Wistar rats. In addition, an increase in the average macrophage diameter was noted following a 90 day exposure (Glaser et al., 1990; Glaser et

al., 1985). In contrast, macrophage diameter in male rabbits exposed to 0.9 mg/m^3 Cr(VI) for 4–6 weeks was not different from that in controls, although the number of macrophages was significantly increased (Johansson et al., 1986b). The inconsistency in effects on BALF macrophages could be related to the differences in study design (i.e., form of chromium administered, animal species and strain, exposure design, endpoint methodology). The ability to synthesize results across studies is limited due to the small number of studies reporting a particular outcome.

Only two studies examined changes in BALF cell populations other than macrophages after inhalation exposure to Cr(VI). Significant increases in the percentage of BALF lymphocytes were observed in Wistar rats after 28 and 90 days of exposure to 0.025 mg/m^3 and 0.05 mg/m^3 Cr(VI). However, after 90 days of exposure at a higher dose (0.2 mg/m^3) the percentage of BALF lymphocytes was not significantly different from control. Similarly, the percentage of BALF granulocytes / neutrophils was significantly increased over control only after exposure to 0.05 mg/m^3 Cr(VI), and decreased compared to control at the higher dose of 0.2 mg/m^3 (Glaser et al., 1985). However, the percentage of BALF granulocytes / neutrophils was demonstrated to significantly increase over time following exposure to 0.36 mg/m^3 Cr(VI) in a different study utilizing F-344 rats (Cohen et al., 2003). The differences in rat strain and exposure levels limit ability to draw conclusions for these other cell populations, but the two studies do demonstrate changes at both lower and higher levels of exposure.

Limited investigation of BAL cells provides equivocal evidence of changes in functional activity of the macrophages. Specifically, no functional changes were observed in macrophages from rabbits exposed to 0.9 mg/m^3 Cr(VI) for 4–6 weeks (Johansson et al., 1986b) based on measures of oxidative metabolic activity (via ability to reduce nitro blue tetrazolium) and phagocytic activity (using fluorescently-labeled yeast cells). However, male Wistar rats exposed to 0.05 mg/m^3 Cr(VI) for 28 days, and to 0.025 mg/m^3 and 0.05 mg/m^3 for 90 days displayed significant increases in phagocytosis of latex particles. Interestingly, at higher concentrations (0.2 mg/m^3) phagocytosis was significantly reduced (Glaser et al., 1985). In addition, exposure to 0.2 mg/m^3 Cr(VI) for 42 days prior and 49 days post challenge with iron oxide particles demonstrated significant reductions in early and late phase clearance (Glaser et al., 1985).

One *medium* confidence study evaluated several biochemical markers of cell injury (Glaser et al., 1990). They reported significant increases in total protein, albumin, and LDH activity in the BALF at all Cr(VI) concentrations in male Wistar rats exposed for both 30 and 90 days (90-day time point, LOAEL 0.05 mg/m^3); increases were concentration-related and were statistically significant at most concentrations investigated. Glaser et al. (1990) also included a group of rats exposed for 90 days with a 30-day recovery period. The author found that many of the BALF endpoints, including total number of macrophages, number of dividing macrophages, and LDH levels, had returned to approximately control values at the end of the recovery period. However, BALF total protein remained statistically significantly elevated at all exposure concentrations, and BALF albumin remained statistically significantly elevated in the two highest concentration groups (0.20

and 0.40 mg/m^3) even after recovery (Figure 3-9). Although only evaluated in one *medium* confidence study, there is additional support for these findings. Zhao et al. (2014) (considered a supplemental study due to use of intratracheal instillation exposure) reported statistically significant increases in albumin and total protein levels in BALF isolated from male Sprague-Dawley rats exposed to 0.022 or 0.22 mg/kg Cr(VI) once per week for four weeks via intratracheal instillation.

Although increases in BALF total protein are characteristic of acute lung injury, this marker alone is considered insufficient to indicate lung injury due to its nonspecific nature and unknown source. BALF protein can increase due to leakage of vascular fluid, and/or lung cells releasing more protein in the alveolar lining fluid. A more specific indicator is the observation of increased BALF albumin, which comprises a major portion of BALF protein. Albumin in BALF can only come from vascular leakage, since lung cells will not make and release albumin to the lumen (Kodavanti, 2014); consequently, increased albumin indicates an alteration in the epithelial and vascular permeability of the lung. While the database that evaluated BALF albumin, protein and LDH only includes one to two studies, the positive evidence suggests lung epithelial and vascular injury following Cr(VI) exposure.

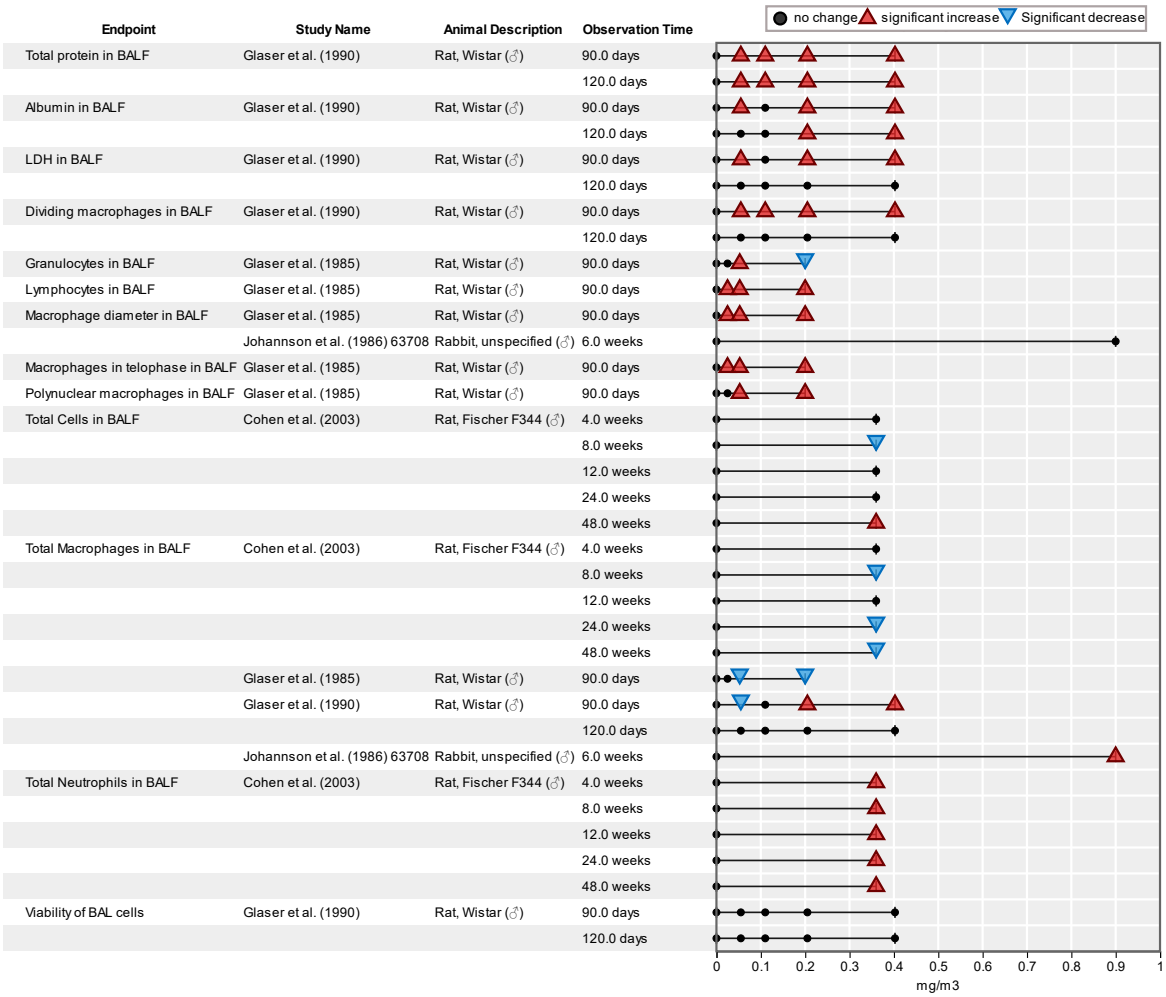

Figure 3-9. Lung cellular responses in BALF in male animals. The 120-day observation time in Glaser et al. (1990) incorporates 90 days of exposure followed by a 30-day period of no exposure (recovery time). Click to see interactive graphic. A graphic containing 30-day data by **Glaser et al. (1990)** can be found in HAWC. An expression of dose-response for selected cellular responses can be found in Section 4.2.1 and in HAWC.

Lung Histopathology

Histopathology is a classic approach used in evaluating effects on the lung and can detect a large range of effects from minor changes in cell populations to significant structural alterations. Seven of the included studies reported histopathological outcomes, comprising five *medium* confidence, one *low* confidence, and one *uninformative* study. Nettesheim et al. (1971) was rated *low* confidence for the outcome of histopathology. Results for this study were only provided qualitatively and without identifying lesions in any specific treatment group or comparison to control (see HAWC for details).

One of the *medium* confidence studies dealt specifically with in vitro ultrastructural electron microscopy of macrophages with no additional tissue characterization (Johansson et al., 1986b). In

general, three of the four remaining *medium* confidence, short-term and subchronic studies of Cr(VI) in rats and rabbits provide consistent evidence of histiocytosis (macrophage accumulation) in the lung (Kim et al., 2004; Glaser et al., 1990; Johansson et al., 1986a) while one subchronic rat study (Glaser et al., 1985) reported normal histopathology findings following Cr(VI) exposure (Figure 3-10).

In one *medium* confidence study, the incidence of accumulation of macrophages in the alveolar and peribronchial region of the lung was increased in male Wistar rats exposed to 0.050–0.40 mg/m^3 Cr(VI) as sodium dichromate for exposure durations of 30 days (incidence: 30%–80%; the concentration-response curve was nonmonotonic, with maximal incidence at 0.10 mg/m^3), 90 days (incidence: 90%–100%), and 90 days with a 30-day recovery period (incidence: 50%–100%) (Glaser et al., 1990). A second *medium* confidence study of similar design by the same authors did not appear to have investigated these effects (Glaser et al., 1985).

Additionally, macrophage aggregation and the accumulation of foamy cells were observed in male Sprague-Dawley rats exposed to Cr(VI) as chromium trioxide aerosol for 90 days Kim et al. (2004). All rodents in the high concentration group (1.25 mg/m^3) exhibited accumulation of macrophage aggregations and foamy cells in the alveolar region. This effect was observed to a lesser extent at 0.5 mg/m^3 but was not observed at 0.2 mg/m^3. This indicates a dose-response relationship; quantitative data for these effects were not presented in this study but the pattern can be inferred based on statements regarding number of animals (i.e., 'all', 'less than all', 'none').

Finally, increased intra-alveolar or intrabronchiolar accumulation of macrophages was reported in 4 of 8 male rabbits exposed to 0.9 mg/m^3 Cr(VI) in the form of sodium chromate for 4–6 weeks (Johansson et al., 1986a). Some macrophages were enlarged, multinucleated or significantly vacuolated and accumulated in a nodular formation. In this study and a companion study that examined macrophages lavaged from the right lung of these rabbits (Johansson et al., 1986b), ultrastructural examination of macrophages revealed large lysosomes with dark or electron-dense patchy inclusions and short membranous fragments or lamellae. The percentage of cells that contained inclusions and the percentage of macrophages with a smooth surface were stated to be significantly increased in the Cr(VI)-exposed group ($p < 0.02$; however, quantitative data were not presented (Johansson et al., 1986b).

Evidence for Cr(VI)-related histopathologic changes in the lungs other than macrophage accumulation is limited, and there is some suggestion of a transient effect. A high incidence of bronchioalveolar hyperplasia (70–100%) was reported in male Wistar rats after 30 days of exposure to 0.050–0.40 mg/m^3 Cr(VI) relative to the control (10%) (Glaser et al., 1990). The same study reported lower incidence of this effect after 90 days of exposure, and after 90 days of exposure with a 30-day recovery period. There was an increased incidence of fibrosis (10–40%) in the groups exposed for 30 days to concentrations at or above 0.1 mg/m^3 Cr(VI), but no increase for the 90-day exposure groups. Glaser et al. (1990) also stated that the upper airways of male Wistar rats exposed 0.1–0.40 mg/m^3 Cr(VI) showed focal inflammation; however, incidence data were not

reported, and the exposure period was not stated. Other investigators did not discuss examination of the upper respiratory tract in experimental animals. Glaser et al. (1985) noted qualitatively that all Wistar male rats exposed for 90 days to 0.025–0.20 mg/m^3 Cr(VI) exhibited normal histologic findings in the lung. Nettesheim et al. (1971) exposed mice to calcium chromate dust from 6 months to approximately 120 weeks at a single concentration of 13 mg/m^3. This concentration was significantly higher than those used in the Glaser et al. studies. The study observed marked changes in the small airways (ranging from epithelial necrosis and atrophy to marked hyperplasia). In addition, the study observed bronchiolarization of the alveoli, and alveolar proteinosis with distention of the terminal bronchioli and alveoli.

In general, histiocytosis and other effects observed in macrophages were observed in the lung following Cr(VI) exposure. Less data were available for bronchiolar hyperplasia, and there is some indication those effects did not persist. The study design by Glaser et al. (1990) allowed for histopathological effects to be observed as a function of concentration and time (including after a recovery period). Bronchiolar hyperplasia peaked at the earliest time point examined (30 days) and diminished over time. Histiocytosis peaked at 90 days and only slightly diminished during the 30-day recovery period. Based on the 30- and 90-day experiments, and the recovery period data, the structural changes in the lung appear to be transient while the influx of cells persists.

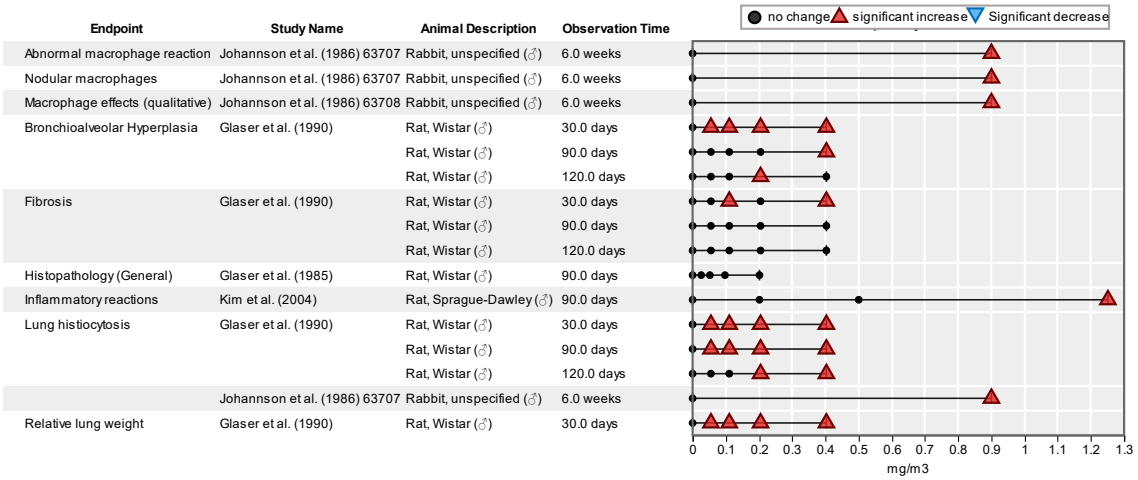

Figure 3-10. Histopathological results and effects in macrophages in male rat lungs. Results from **Kim et al. (2004)** were qualitative, and dose ranges and the noted statistically significant dose groups are presented here for comparative purposes. The 120-day observation time from **Glaser et al. (1990)** incorporates 90 days of exposure followed by a 30-day period of no exposure (recovery time). Click to see interactive graphic. A figure containing incidence data of selected histopathological outcomes can be found in Section 4.2.1 or in HAWC.

Lung Weight

Increases in lung weight, a nonspecific indicator of lung injury, can occur from a variety of pulmonary conditions, including edema, inflammation (including macrophage accumulation),

fibrosis, accumulation of foreign matter, or abnormal tissue growth (e.g., tumors). Changes in lung weight were examined in five of the included studies, one of which was *medium* confidence while the remaining four were considered *low* confidence for this endpoint.

The relative lung weight outcome in Glaser et al. (1990) was rated as *low* confidence because the study lacked sufficient methodological details for measuring lung weight and reduced body weight gain in exposed rats. The relative lung weight outcome in Glaser et al. (1986) was rated as *low* confidence because the study lacked sufficient methodological details for measuring lung weight, only included data for the high dose group, and did not report absolute lung weight (despite reporting end-of-study body weight loss). The lung weight outcome in Johansson et al. (1986a) was rated *low* confidence for several reasons: inconsistent exposure times on study, variable weight/age of animals in the control and exposure groups, lack of documentation of end-of-study weight, and reporting of absolute lung weight only. The Kim et al. (2004) study was also rated *low* confidence for lung weight due to reporting of only relative weights, when both relative and absolute weights of the lung and other organs are preferred for assessing effects from body weight changes and differing types of lung toxicity.

Increased lung weight, which was attributed to accumulation of macrophages, was observed in one *medium* confidence and one *low* confidence study following subchronic inhalation exposure to Cr(VI). Glaser et al. (1985), reported increased mean relative lung weights (9–35%) in Wistar rats exposed for 90 days to Cr(VI) at concentrations of 0.05–0.20 mg/m^3. Study authors also noted that relative lung weights were also increased after 28 days of exposure to Cr(VI) concentrations ≥0.05 mg/m^3; however, quantitative lung weight data were not presented for these higher doses. In a similarly designed study by the same investigators, Glaser et al. (1990) reported a concentration-dependent increase in relative lung weight in Wistar rats following both 30 and 90 days of exposure (9–48%), and following a 90-day exposure with a 30-day recovery period (5–23%); the increase was statistically significant at concentrations of 0.10–0.40 mg/m^3 at all time points, and at the lowest concentration (0.05 mg/m^3) after 30 days of exposure. In contrast, statistically significant changes in lung lower left lobe weight were not observed in male rabbits exposed to 0.9 mg/m^3 for 4–6 weeks (Johansson et al., 1986a), and changes in relative lung weight were not observed in male Sprague-Dawley rats exposed at concentrations ranging from 0.2–1.25 mg/m^3 for 90 days (Kim et al., 2004).

In the only available chronic study (Glaser et al., 1986), mean relative lung weight in Wistar rats exposed to 0.10 mg/m^3 (highest concentration tested) for 18 months and kept on study for another 12 months (total time on study: 30 months) was 15% greater compared with controls, although this change cannot be interpreted as clearly due to macrophage accumulation given the observation of lung tumors at this concentration. Lung weights were not reported for the low- and mid-concentration exposure groups where tumors did not develop, but no changes were noted by the study authors.

To summarize, although there were some inconsistencies in the evidence, increases in lung weights in Wistar rats were observed in the only *medium* confidence study available and a second *low* confidence study by the same authors (Figure 3-11). These changes in lung weight may represent an indicator of nonspecific lung injury or inflammation associated with Cr(VI) inhalation. The studies reveal that changes in lung weight may vary by species, strains, and exposure duration and may attenuate over time.

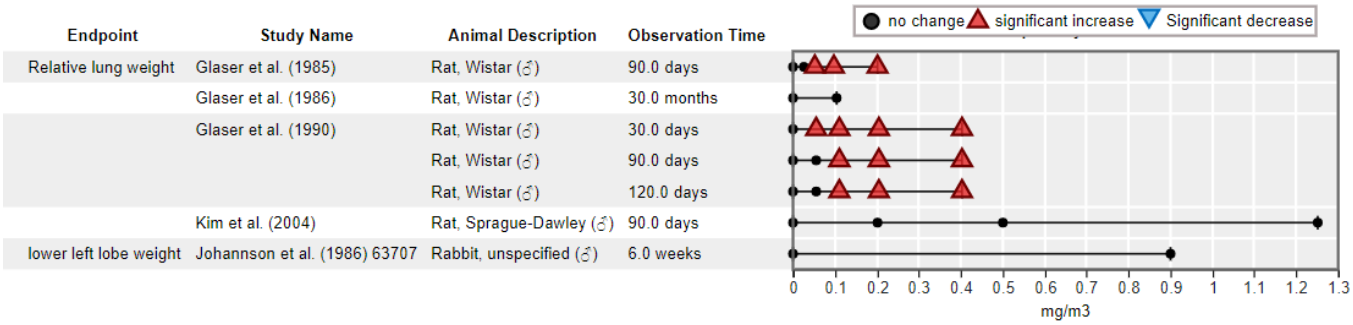

Figure 3-11. Lung weight in male animals. The 120-day observation time incorporates 90 days of exposure followed by a 30-day period of no exposure (recovery time). Click to see interactive graphic.

Other Findings

Various clinical findings that could be related to either upper or lower respiratory tract effects were observed in two studies. Obstructive respiratory dyspnea was reported in male Wistar rats exposed for 30 days to 0.2 or 0.4 mg/m^3 Cr(VI) in a 30 and 90-day study, although data were not provided regarding incidence, severity, persistence or recovery (Glaser et al., 1990). This may indicate hypersecretion of mucus and accumulation in the upper respiratory tract (Kodavanti, 2014). In a 13-week inhalation study (Kim et al., 2004), "peculiar sound during respiration" was observed from weeks 1–7 in male Sprague-Dawley rats exposed to 0.2–1.25 mg/m^3 Cr(VI) in the form of chromium trioxide aerosol mists.

Summary of Lower Respiratory Effects in Animals

Based on the evidence presented above, BALF parameters were the most sensitive indicators of potential lung injury by chromium exposure, which were observed in multiple studies of medium confidence in rats and rabbits. These studies typically exposed laboratory animals to aqueous aerosols of Cr(VI) (with the exception of Nettesheim et al. (1971) which used dust and was a low confidence study). Thus, the effects observed in *medium* confidence studies were unrelated to particle response[24]. Increases in BALF total protein, albumin, and lactate dehydrogenase (LDH) activity are characteristic of acute lung injury. While total protein is a nonspecific indicator, the

[24]For control groups, studies typically exposed rodents to filtered air or inert aerosols (with diluent likely being sterile water, although none of the articles provided details). Neither of these are expected to have adverse effects on the airways.

concentration of albumin in the BALF is normally very low, and an increase indicates an alteration in the epithelial and vascular permeability of the lung. Damage to cells releases the cytosolic enzyme LDH; increased enzymatic activity of LDH in the BALF is a common finding with acute lung injury (Henderson et al., 1985). The increase in BALF albumin and LDH activity provide evidence of lung injury following Cr(VI) exposure via inhalation; however, it should be noted that this evidence came from a single study, and no other studies examined these effects. These findings were accompanied by some evidence of histiocytosis (macrophage accumulation) and increased leukocytes in plasma (see Section 3.2.6), which are supportive of inflammatory lung responses (Nikula et al., 2014), although these findings generally lessened with longer chromium exposure durations and may reflect adaptation or resolution of the cellular responses during these later time points of exposure.

The evidence base of histopathological effects in the lung were mostly limited to macrophage accumulation, which were observed by multiple studies of *medium* quality. Findings for other histopathological changes, such as bronchioalveolar hyperplasia, were only reported in one study.

Increased lung weight was observed in the single *medium* confidence study in Wistar rats, but not in lower confidence studies in other species and strains. However, lung weight is a nonspecific indicator of lung injury and may be a consequence of multiple other more sensitive outcomes (such as increased macrophages).

3.2.1.3. *Mechanistic Evidence*

Mechanistic evidence indicating the biological pathways involved in respiratory toxicity following the inhalation of Cr(VI) is summarized below. Studies of human occupational inhalation exposures, in vivo studies in mammals that were exposed via inhalation or intratracheal instillation, and in vitro studies in human primary or immortalized lung cells were prioritized for informing interpretations of respiratory health effects following inhalation exposure to Cr(VI) in humans; this prioritization strategy is summarized in Appendix Table C-31. These studies focused primarily on oxidative stress and cellular toxicity of the lung; more detailed summaries can be found in Appendix Table C-32.

Oxidative stress

Cr(VI) compounds are strong oxidizers and can readily enter cells, where they interact with intracellular reductants to form Cr(VI) intermediate species [Cr(V) and Cr(IV)] and the stable Cr(III). These intermediate species form reactive oxygen species (ROS) that at high levels can damage intracellular components, including DNA. Increased oxidative stress induced by Cr(VI) has been consistently reported in many species and cell types (reviewed in Cancer, Section 3.2.3)

Twenty-two observational studies measuring various indicators of oxidative stress in industrial workers exposed to Cr(VI) were identified; 21 of these detected systemic biomarkers of oxidative damage in urine and/or blood and are summarized in Appendix Section C.3.6. While a few

occupational exposure studies did not detect statistically significant indicators of oxidative stress in exposed workers (Wultsch et al., 2014; Pournourmohammadi et al., 2008; Kim et al., 1999; Faux et al., 1994; Gao et al., 1994), most studies reported statistically significant increased incidences of oxidative stress through increased levels of relevant markers (e.g., 8-OHdG adducts, lipid peroxidation, decreased levels of antioxidant enzymes) that correlated with exposure to Cr(VI) in urine and blood (El Safty et al., 2018; Hu et al., 2018; Yazar and Yildirim, 2018; Pan et al., 2017; Mozafari et al., 2016; Elhosary et al., 2014; Zendehdel et al., 2014; Wang et al., 2012b; Zhang et al., 2011; Kalahasthi et al., 2006; Goulart et al., 2005; De Mattia et al., 2004; Maeng et al., 2004; Kuo et al., 2003; Huang et al., 1999; Gromadzińska et al., 1996). One group investigated welders exposed to Cr(VI), finding significant upregulation of a glycoprotein, Apolipoprotein J/Clusterin, that correlated with chromium levels in blood and urine; ApoJ/CLU has been shown to be involved in cellular senescence and is implicated in diseases related to oxidative stress, inflammation, and aging (Alexopoulos et al., 2008).

Less evidence is available for oxidative stress measured in the lung, summarized in Appendix Section C.2.1. One study in exposed workers, Kim et al. (1999), analyzed respiratory epithelial cells from exposed lead chromate pigment factory workers and did not detect a difference in 8-OHdG levels compared to office workers in the same factory. However, the chromium levels measured in the blood were similar between the exposed and referent groups, indicating that perhaps exposure misclassification could have contributed to the null findings. In animals, Maeng et al. (2003) exposed rats via inhalation to 0.18 or 0.9 mg/m^3 sodium chromate for 1, 2, or 3 weeks and reported increased formation of 8-OHdG adducts after 1 week exposure that resolved at weeks 2–3, despite consistently diminished activity of the enzymes that repair these lesions at weeks 1–3. These results are supported by two studies exposing rats to Cr(VI) via intratracheal instillation that detected significantly increased oxidative DNA lesions (8-OHdG) in the lung following four weekly intratracheal instillations of 0.063 or 0.630 mg Cr/kg (Zhao et al., 2014) or once daily administrations of 0.09 mg Cr(VI)/kg for three consecutive days (Izzotti et al., 1998).

Inhalation exposures provide a direct route for Cr(VI) compounds to be absorbed by the bronchial epithelium, and increased oxidative stress induced by Cr(VI) has been confirmed in studies of human lung cells. Cells deficient in the ability to repair oxidative DNA lesions were reported to have a significant increase in cytotoxicity and cell cycle delay following Cr(VI) exposure (Reynolds et al., 2012; Reynolds and Zhitkovich, 2007). Cr(VI) exposure has also been observed to cause oxidative stress with minimal or no cytotoxicity, indicating that oxidative stress may in some instances be induced at levels that do not affect cell viability. Caglieri et al. (2008) noted increased lipid peroxidation in BEAS-2B human bronchial epithelial cells with cytotoxicity but also in A549 human lung adenocarcinoma cells at subtoxic levels. Asatiani et al. (2011; 2010) observed increased ROS and the antioxidant enzymes glutathione peroxidase, glutathione reductase, and catalase at transiently toxic Cr(VI) concentrations. Martin et al. (2006) found that adding glutathione to Cr(VI)-treated cells decreased levels of ROS; conversely, addition of ascorbate

(Vitamin C), a primary intracellular reducer of Cr(VI), increased levels of ROS. The authors theorize that the ascorbate reduction pathway could interact with reactive Cr(V) intermediates that are generated via the glutathione pathway, stabilizing Cr(V) and leading to more potential interaction between Cr(V) and intracellular components. In addition, ascorbate reduction of Cr(VI) occurs at a much faster rate than glutathione and has been shown to result in higher levels of genotoxicity than glutathione (Zhitkovich, 2011). Another group reported that cellular thioredoxins and peroxiredoxins are especially sensitive to oxidation by Cr(VI), disrupting redox signaling and affecting cell survival (Myers et al., 2011; Myers et al., 2010; Myers and Myers, 2009; Myers et al., 2008).

Cytotoxicity

Apoptosis, or programmed cell death, typically plays a protective role in eliminating damaged cells from the body but can also be triggered by excessive levels of ROS, contributing to tissue damage and inflammation. The evidence from studies of exposed workers for specific measures of apoptosis is sparse due to inadequate information to characterize Cr(VI) exposures. Gambelunghe et al. (2003) did not detect an increase in apoptosis among chrome-plating workers, although this study was estimating cell death using the comet assay, which is an insensitive method of measuring apoptosis. Wultsch et al. (2017) reported increased cytotoxicity in the exfoliated buccal and nasal cells of electroplaters indicated by histopathological evidence of nuclear anomalies consistent with apoptosis; however, this study was evaluated for another nuclear effect, micronuclei (Section 3.2.3.2), and was found to be *uninformative* due to critical deficiencies in the exposure domain. Halasova et al. (2010) determined that expression of the apoptosis inhibitor survivin protein was decreased and pro-apoptotic p53 was increased in former chromium workers with lung cancer compared to unexposed lung cancer patients, but the authors did not describe methods for exposure assessment and characteristics of the exposed and unexposed groups that may also affect the apoptosis measures were not compared. In animal models, one intratracheal instillation exposure study in rats observed increased apoptosis in bronchial epithelium and lung parenchyma (D'Agostini et al., 2002).

Cytotoxicity occurring at micromolar Cr(VI) levels that increases with dose and duration of exposure has been consistently observed in numerous in vitro studies in human lung cells (Yang et al., 2017; Reynolds et al., 2012; Asatiani et al., 2011; Asatiani et al., 2010; Caglieri et al., 2008; Reynolds and Zhitkovich, 2007; Martin et al., 2006; Pascal and Tessier, 2004; Carlisle et al., 2000; Popper et al., 1993), with some studies specifically detecting increases in apoptotic cell death (Reynolds et al., 2012; Azad et al., 2008; Reynolds and Zhitkovich, 2007; Gambelunghe et al., 2006; Carlisle et al., 2000). Evidence for the involvement of a p53-mediated pathway for the induction of apoptosis was conflicting; Carlisle et al. (2000) observed a 4–6 fold increase in p53 in LL-24 human lung fibroblasts, and Gambelunghe et al. (2006) observed increased expression of p53 in MOLT-4 lymphoblastic leukemia cells, but a similar increase in p53 was not observed in BEAS-2B human

bronchial epithelial cells, and Reynolds and Zhitkovich (2007) determined that p53 status had no effect on apoptosis (or cytotoxicity) in primary human lung IMR90 fibroblasts or H460 human lung epithelial cells. Similarly, information on the identification of caspases involved in Cr(VI)-induced apoptosis was conflicting, with one group reporting that inhibiting caspase-3, -8 and -9 did not reduce apoptosis in MOLT-4 lymphoblastic leukemia cells (Gambelunghe et al., 2006), while another group reported a significant decline in apoptosis after specific suppression of caspase-9 in H460 human lung epithelial cells (Azad et al., 2008). Autophagy, another cellular defense mechanism that can alternately induce or suppress cell death, was reported following Cr(VI) exposure in A549 human lung adenocarcinoma cells (Yang et al., 2017). The autophagy was correlated with a transcription factor, HMGA2, that is highly expressed in lung cancer patients, and was suppressed by silencing HMGA2.

Cytotoxicity appeared to be dependent on cell type, possibly reflecting underlying differences in sensitivity, with A549 lung adenocarcinoma cells slightly more resistant to cytotoxicity than BEAS-2B bronchial epithelial cells derived from non-tumorigenic cells. Asatiani et al. (2011) observed that at doses ≤5 μM, the cytotoxicity in HLF fetal human lung fibroblasts and L-41 human epithelial-like cells resolved after 24 h, but these concentrations were sufficient to induce oxidative stress and an upregulation of antioxidant enzymes. Increasing levels of ascorbate to better simulate physiological levels, were found to potentially increase oxidative damage (Martin et al., 2006) or promote cytotoxicity and apoptosis by forming Cr-DNA adducts (Reynolds et al., 2012; Reynolds and Zhitkovich, 2007; Carlisle et al., 2000). This evidence implies that the pathways for Cr(VI)-induced apoptosis and toxicity in human lung cells are complex and likely to differ substantially among species and cell type.

Lung cellular inflammation

Specific support for the lung cellular responses in animals discussed in the above evidence synthesis is also provided by two supplemental studies in animals that did not meet PECO criteria due to the route of exposure used (intratracheal instillation). Zhao et al. (2014) reported statistically significant increases in relative lung weight and in albumin and total protein levels in BALF isolated from male Sprague-Dawley rats exposed to 0.063 or 0.630 mg Cr(VI)/kg once per week for four weeks via intratracheal instillation. These effects were concurrent with increases in oxidative damage (8-OHdG lesions) and NF-κB, consistent with oxidative stress and inflammation. In another study in rats exposed to 0.0035, 0.017, or 0.087 mg Cr(VI)/kg, 5x/week, or 0.017, 0.087, or 0.44 mg/kg, 1x/week via intratracheal instillation for 30 weeks, lungs of animals dosed with ≤0.087 mg/kg Cr(VI) contained macrophage foci, while in the high dose group, in addition to benign and malignant tumors, severe damage and fibrosis to the bronchioloalveolar region of the lung was observed, alongside inflammatory foci that included alveolar macrophages, epithelial cell proliferation, and inflammatory thickening of the alveolar septa (Steinhoff et al., 1986).

Studies investigating immune toxicity (Section 3.2.6) have observed changes in various cytokine signaling in the blood, serum, and plasma of chromate workers exposed to Cr(VI) (Qian et

al., 2013; Mignini et al., 2009; Kuo and Wu, 2002) (summarized in Appendix Table C-38), although one study specific to the lung in rats exposed via inhalation to 0.119 mg Cr(VI)/m^3 for 5 h/d for 5 consecutive days reported no detectable changes in several cytokines in BALF (Cohen et al., 2010). In human lung cells in vitro, cytotoxicity was shown to correlate with a net loss of urokinase-type plasminogen activator activity that has been shown to promote pulmonary fibrosis (Shumilla and Barchowsky, 1999), as well as an inflammatory response via protein phosphorylation and cytokine signaling (Pascal and Tessier, 2004). Although the direction of these changes was not consistent across studies, fluctuations in systemic cytokine levels and redox imbalance are characteristic of an inflammatory response and may be indicative of a disruption in the regulatory balance that dictates normal immune system function.

3.2.1.4. *Integration of Evidence*

Overall, the available **evidence indicates** that Cr(VI) likely causes lower respiratory tract effects in humans under relevant exposure scenarios (see Table 3-7). Cr(VI) is a known lung carcinogen, but the evidence for noncancer effects in the respiratory tract (with the exception of nasal effects) is more sparse. This evidence integration conclusion is based on observations of decreased lung function among chromium-exposed workers in two of the four *low* confidence human studies and of biochemical effects indicative of lung injury (albumin, LDH, and total protein in BALF) in *medium* confidence animal studies, supported by supplemental and mechanistic observations consistent with an inflammatory tissue response following Cr(VI) exposure.

The development of the ATS guidelines in 1987 greatly increased the reliability of spirometry measurements. These improvements to outcome measurement technology and methods coincide with or came after changes to industrial processes aimed at reducing Cr(VI) exposures in workers. Thus, while researchers were in a better position to reduce outcome measurement error after the ATS guidelines become available, at the same time, the contrast in exposures was reduced compared to previous decades, impacting study sensitivity. All four of the included human studies thus had potential for decreased sensitivity due to lower exposure levels attributed to industrial hygiene and process changes in more recent years. All four included human studies were found to be *low* confidence, and two of these reported decreases in lung function in chromate workers compared to referents (Li et al., 2015b; Kuo et al., 1997b). Given the consistency of the findings from these two *low* confidence studies and biological plausibility provided by supporting evidence for changes in inflammatory, oxidative stress, and cytotoxicity biomarkers in workers exposed to Cr(VI) (described under "Mechanistic Evidence"), the human studies are interpreted to provide *slight* evidence for lower respiratory tract effects.

The pathogenesis of chronic pulmonary disease induced by chemicals toxic to the lung involves the accumulation of inflammatory macrophages (Laskin et al., 2019). In the available animal studies, which together provide moderate evidence of lung inflammation, histopathological changes in the lung following Cr(VI) exposure included histiocytosis (macrophage accumulation) observed in four out of the five *medium* confidence animal studies. Infiltration of histiocytes was

also observed in multiple other organs following oral exposure in rodents (see a broader discussion in Section 3.2.6, Immune Effects), which increases confidence that this inflammatory effect is a result of Cr(VI) exposure. For inhalation exposure, histiocytosis was biologically significant because it accompanied markers in bronchoalveolar lavage fluid (BALF), and increased leukocytes in plasma (see Section 3.2.6), which are observations supportive of inflammatory lung responses (Nikula et al., 2014). Cellular responses consistent with injury in the lung following Cr(VI) exposure were also observed in animal studies, including increased albumin, total protein, and LDH activity in BALF, biomarkers known to be evidence of injury and vascular leakage in the lower airway and deep lung (Kodavanti, 2014). Additionally, findings of increased lung weights in a single study of Wistar rats (but not other strains or species examined in lower confidence studies) and clinical findings in two rodent studies of obstructive respiratory dyspnea (Glaser et al., 1990) and "peculiar sound during respiration" and periodic nose bleeds (Kim et al., 2004), are coherent with the inflammatory changes consistently indicated in the available animal studies.

As described in Section 3.1, inhaled chromium can accumulate in high concentrations at portal-of-entry tissues (such as the respiratory epithelium), resulting in absorption into the epithelial cells in the lung and lung airways, and particles may accumulate in susceptible areas such as airway bifurcation sites. Studies investigating the underlying mechanisms involved in Cr(VI)-induced lung toxicity report significant cytotoxicity at micromolar concentrations in vitro, concurrent with indications of an inflammatory response (oxidative stress, cytokine and nuclear transcription factor activation) as well as increased programmed cell death (apoptosis, autophagy) in response to Cr(VI) exposure. These data support the biological plausibility of the inflammatory tissue responses observed in Cr(VI)-exposed animals. Although the available mechanistic studies in humans were measuring systemic markers of oxidative stress and inflammation in the blood and urine rather than specifically in the lung, consistent evidence of increased reactive oxygen species generation and cytokine modulation in exposed workers is consistent with an inflammatory response that contributes to health effects.

For lower respiratory tract effects, there were inconsistencies in the data that may be explained by differences in study design and particle size. Large inhaled particles (with diameter >5 μm) will deposit in the extrathoracic region, particles greater than 2.5 μm are generally deposited in the tracheobronchial regions, and particles less than 2.5 μm are generally deposited in the pulmonary region (OSHA, 2006). The rodent study of sodium dichromate aerosols by Glaser et al. (1990; 1985) likely induced effects in the lower respiratory tract due to the small particle sizes achieved by the experiment (MMAD < 0.4 μm). For the human occupational studies, particle sizes may have been larger and more variable (Kuo et al., 1997a), causing a lower proportion of Cr(VI) to deposit in the pulmonary region. However, human studies of occupationally exposed workers still provide some evidence for pulmonary function deficits with increased Cr(VI) exposure. Animal and human studies also differed with respect to the types of data collected, which precluded the ability to directly compare effects. Human data were based on functional measures (pulmonary function

evaluated using spirometry), whereas animal data were based on histopathological measures and cellular responses. The endpoints reported by studies in humans and animals were complementary; overall the currently available **evidence indicates** that Cr(VI) is likely to cause lower respiratory toxicity in humans.

Table 3-7. Evidence profile table for respiratory effects other than cancer

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from studies of exposed humans					⊕⊕⊙ The **evidence indicates** that Cr(VI) inhalation is likely to cause lower respiratory toxicity in humans, based on *moderate* evidence in rats showing increases in biochemical indicators of lung injury and evidence of lung inflammation. This is supported by *slight* human evidence of decreased pulmonary function from *low* confidence studies of exposed workers and supportive mechanistic evidence for increases in oxidative stress and cytotoxicity biomarkers. The findings in animals are consistent with known biomarkers of human pulmonary dysfunction and thus considered relevant to humans. The **evidence is inadequate** to determine whether oral Cr(VI) exposure might be capable of causing noncancer respiratory effects. No respiratory effects were observed following ingestion. As described in Section
PULMONARY FUNCTION **Four *low* confidence studies in occupationally exposed adult workers:** Kuo et al. (1997b) Li et al. (2015b) Lindberg and Hedenstierna (1983) Sobaszek et al. (1998)	Exposure to Cr(VI) was associated with decreased FVC and FEV1.0 in two *low* confidence studies (association not statistically significant for FVC in one of the two studies). No association between Cr(VI) and FVC, FEV1.0, or FEV1/FVC was found in the remaining *low* confidence studies, which may be explained by low study sensitivity.	• Coherence of observed effects on multiple measures of pulmonary function (apical studies) • Observed decrements in FEV and FVC in two low confidence studies	• Imprecision of effect estimates • Low confidence studies	⊕⊙⊙ *Slight* Based on decreased pulmonary function with higher exposure to Cr(VI) in two low confidence studies.	
Evidence from animal studies					
LUNG CELLULAR and BIOCHEMICAL RESPONSES, including HISTOPATHOLOGY **Six *medium* confidence studies in rats and rabbits:** Kim et al. (2004) Cohen et al. (2003) Glaser et al. (1985) Glaser et al. (1990) Johansson et al. (1986a) Johansson et al. (1986b) **One *low* confidence study in mice:**	Inflammatory changes in BALF Increased macrophages in two *medium* confidence studies, but no changes or slight decreases in two others. Increases in neutrophils/ granulocytes in two *medium* confidence studies, and increased lymphocytes up to 90 days in one *medium* confidence study. Macrophage Functional changes Increased phagocytosis in one *medium* confidence study (at	• Consistent evidence of inflammatory changes across *medium* confidence studies and rat strains • Coherence of observed effects across different biomarkers of lung injury	• Biomarker evidence of lung injury is less specific than pathology • Lack of duration-dependence (some effects weakened with longer exposures) • Some unexplained inconsistency in	⊕⊕⊙ *Moderate* Coherent and largely consistent increases in biomarkers of pulmonary injury and inflammatory cells in BALF and lung tissue, as well as mechanistic findings supportive of	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Nettesheim et al. (1971)	concentrations ≤0.05 mg/m^3), but no change in another. BALF Biochemistry Increased protein, albumin and LDH in one *medium* confidence study. Histiocytosis Four of five *medium* confidence studies reported the accumulation of macrophages in the lung by histopathology. Other Histological Changes Mixed evidence for bronchiolar hyperplasia (one *medium* confidence study); epithelial hyperplasia, atrophy, and necrosis (one *low* confidence study); and normal histopathology (one *medium* confidence study).	• Concentration-response gradient for most effects • Large effect magnitude for histopathological effects • Biological plausibility (mechanistic evidence of lung oxidative stress and apoptosis in animal models, primarily from instillation and in vitro studies)	findings for macrophages in BALF and their functional changes • Unclear adversity of some inflammatory changes and lack of expected coherence with more overt histopathological markers of injury	inflammatory changes in lung.	3.1, Cr(VI) can expose portal-of-entry tissues, and reduction of Cr(VI) in these tissues and red blood cells decreases uptake by other organ systems.
LUNG WEIGHT One *medium* confidence study in rats: Glaser et al. (1985) Four *low* confidence studies in rats and rabbits: Glaser et al. (1986) Kim et al. (2004) Glaser et al. (1990) Johansson et al. (1986a)	Lung Weight Increased lung weights were reported in the only *medium* confidence study and one *low* confidence study, both in Wistar rats, with exposures for up to 90 days and for 18 months; however, effects were not observed in other *low* confidence studies of male rabbits exposed for 4–6 weeks or	• Concentration-response gradient in two studies (Glaser et al. (1990; 1985)) • Effect magnitude (up to 48% increased relative lung weight) • Biological plausibility	• Some inconsistency across studies, although inconsistent studies were *low* confidence	⊕⊙⊙ *Slight* Changes in lung weight were reported in one rat strain but not in *low* confidence studies of a different strain or in rabbits.	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	male Sprague-Dawley rats exposed for 90 days.	(increased macrophages will increase lung weight)			
Mechanistic evidence					
Biological events or pathways	**Summary of key findings and interpretations**			**Judgments and rationale**	
Oxidative stress	*Interpretation:* Inhalation exposure to Cr(VI) induces a disruption of the cellular redox balance in the lung that is a key component of Cr(VI)-induced lung toxicity. *Key findings:* • Consistent evidence of significant increases in oxidative stress in 17 studies of workers exposed to Cr(VI) that correlated with levels of Cr(VI) in urine and blood • Increased formation of 8-OHdG DNA adducts in one study of rats exposed to Cr(VI) via inhalation • In vitro evidence of oxidative stress with exposure to Cr(VI), including increased ROS production, oxidation of lipids and proteins, and increased antioxidant enzyme activity, in human primary and immortalized lung cells • Deficiency in DNA repair of 8-OHdG lesions led to increased cytotoxicity and cell cycle delay following Cr(VI) exposure in vitro			Biologically plausible, consistent, and coherent observations of oxidative stress, leading to cytotoxicity and possibly involving inflammation, which are interrelated processes involved in cellular stress signaling that can underlie the respiratory effects reported in humans and in animals exposed to Cr(VI). Fluctuations in	
Cytotoxicity	*Interpretation:* Inhaled Cr(VI) is presumed to be cytotoxic to portal-of-entry tissues; this toxicity, primarily shown by one study in animals and multiple studies of human cells in vitro, may involve programmed cell death in the lung. *Key findings:* • Increased apoptosis in the lung of rats exposed to Cr(VI) via intratracheal instillation in one study				

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	• Consistent in vitro evidence of dose- and time-dependent increases in apoptosis following Cr(VI) exposure in human lung cells • Some evidence of increased p53 (which can be pro-apoptotic) with Cr(VI) exposure in humans or human lung cells in vitro			cytokine levels and redox imbalance are characteristic of an inflammatory response and may be indicative of a disruption in the regulatory balance that dictates normal immune system function.	
Inflammation	*Interpretation*: Inflammation induced by inhalation exposure to Cr(VI) may involve pro-inflammatory cytokine signaling and enhanced ROS generation. *Key findings*: • Supplemental evidence of inflammatory cellular changes, histopathology, and increased lung weight in Cr(VI) animal intratracheal instillation studies support animal evidence judgments; these effects were concurrent with increases in oxidative stress and inflammatory cell signaling • Cytokine signaling changes in chromate workers (Appendix C.2.5)				

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3.2.2. Gastrointestinal Tract Effects Other Than Cancer

Studies of the GI tract following ingestion of Cr(VI) in humans and animals have generally reported an increased incidence in nonneoplastic lesions in the stomach and portions of the small intestine. The GI tract is responsible for the digestion, absorption, and excretion of ingested substances. The main function of the stomach is storage and digestion; it is lined with epithelial cells with tight junctions that lack the absorptive villi found in the intestines. In the small intestine, the villi in the semipermeable mucosa consist of epithelial cells characterized by a brush border of microvilli that further increase absorptive capacity. Between the villi are deep cavities called crypts. Both crypts and villi contain epithelial enterocytes and goblet cells that secrete mucus. A schematic of the epithelial morphologies of the stomach and small intestine is provided in Section 3.1.1 Pharmacokinetics, Figure 3-5. While the small intestine has a large absorptive capacity it also serves as a barrier that prevents potentially toxic substances in the lumen, including bacteria, from entering systemic circulation (e.g., by mucus secretion). The crypts in the small intestine supply rapidly dividing stem cells for the renewal of the intestinal epithelium, which turns over within days (Potten et al., 2009; Potten et al., 1997). Within the stomach, gastric stem cells are located within glandular pits, and unlike the small intestine, they are nearer to the lumen and more likely to be exposed to surface irritants (Mills and Shivdasani, 2011). In animal studies, the areas of the small intestine that are more proximal to the stomach (the duodenum and jejunum) appear to be more susceptible to injury than the ileum, the distal portion.

3.2.2.1. *Human Evidence*

The literature search for this assessment did not identify epidemiological studies that met PECO criteria for this health effect. The ATSDR Toxicological Profile (ATSDR, 2012) describes multiple case reports of deaths among adults and children resulting from ingesting Cr(VI) compounds and subsequent damage to the GI tract and other organs. GI effects reported in acute oral poisoning studies identified in the literature search for this assessment include stomach and esophageal pain, diarrhea, lesions of the stomach and duodenum, hemorrhage of the GI tract, and gut mucosal necrosis (Goullé et al., 2012; Baresic et al., 2009; Hantson et al., 2005; Kolacinski et al., 2004; Sharma et al., 2003; Stift et al., 2000; Kołaciński et al., 1999; Loubières et al., 1999; Stift et al., 1998; Kurosaki et al., 1995; van Heerden et al., 1994). The ATSDR Toxicological Profile (ATSDR, 2012) also describes reports of stomach pain, GI ulcer, and gastritis among workers employed in electroplating and chromate production were also described from studies published from 1950–1978. The exposures could have occurred via both inhalation and ingestion of Cr(VI) dusts in the workplace. ATSDR concluded that these studies included no or inappropriate comparison groups and therefore a direct association between Cr(VI) exposure and these signs and symptoms could not be drawn.

3.2.2.2. *Animal Evidence*

Study evaluation summary

Table 3-8 summarizes the four animal bioassays that were considered in the evaluation of noncancer effects in the GI tract from ingested Cr(VI). The studies, conducted by two organizations, the US National Toxicology Program (NTP) (NTP, 2008, 2007) and ToxStrategies, Inc. (Thompson et al., 2012b; Thompson et al., 2011), exposed mice and rats of both sexes to Cr(VI) in drinking water, and were of subchronic duration except for the NTP (2008) 2-year bioassay. Results in all studies were limited to histopathological observations and mechanistic evidence; the latter is also described with the evidence for GI tract cancer in Section 3.2.3.2.

Table 3-8. Summary of included studies for Cr(VI) GI histopathological outcomes and overall confidence classification. Click to see interactive data graphic for rating rationales.

Author (year)	Species (strain)	Exposure design	Exposure route	Histopathology
NTP (2007)	Rat (F344/N), male and female; Mouse (B6C3F1, BALB/c, C57BL/6), male and female	Subchronic	Drinking water	H
NTP (2008)	Rat (F344/N), male and female; Mouse (B6C3F1), male and female	Chronic	Drinking water	H
Thompson et al. (2011)	Mouse (B6C3F1), female	Subchronic	Drinking water	H
Thompson et al. (2012b)	Rat (F344), female	Subchronic	Drinking water	H

High (H), medium (M), low (L), or uninformative (U).

Synthesis of evidence in animals

All four *high* confidence studies in rats and mice reported various histological effects in the GI tract associated with oral exposure to Cr(VI). In the small intestine these included diffuse epithelial/crypt cell hyperplasia, histiocytic cellular infiltration, and degenerative changes in the villi (vacuolization, atrophy, and apoptosis); in the glandular stomach these included squamous metaplasia and gastric ulceration (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007). Across studies, the most commonly observed nonneoplastic GI lesion was epithelial cell hyperplasia in the mouse small intestine (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007). Results from studies in mice and rats are summarized in Figures 3-12 and 3-13, and study design differences are outlined in Table 3-9 (detailed results are summarized in Appendix Table C-32). Dose-dependent histiocytic infiltration, described by NTP (2008) as being of unknown biological significance, was also observed in the small intestine of exposed animals across studies, sexes, and species.

Table 3-9. Design features of studies that examined GI tract effects via the oral route of exposure

Study reference	Species/strain and sex	Exposure duration	Number of animals/group	Dose groups (mg Cr(VI)/kg-d)
NTP (2008)[a]	B6C3F1 mouse, male and female	2 years	50	0, 0.450, 0.914, 2.40, 5.70 (M) 0, 0.302, 1.18, 3.24, 8.89 (F)
NTP (2008)	F344 Rat, male and female	2 years	50	0, 0.200, 0.760, 2.10, 6.07 (M) 0, 0.248, 0.961, 2.60, 7.13 (F)
NTP (2007)	F344 Rat, male and female	90 days	10	0, 1.74, 3.14, 5.93, 11.2, 20.9 (M) 0, 1.74, 3.49, 6.28, 11.5, 21.3 (F)[a]
NTP (2007)	B6C3F1 mouse, male and female	90 days	10	0, 3.1, 5.3, 9.1, 15.7, 27.9 (M+F)
NTP (2007)	B6C3F1 mouse, male	90 days	5	0, 2.8, 5.2, 8.7
NTP (2007)	BALB/c mouse, male	90 days	5	0, 2.8, 5.2, 8.7
NTP (2007)	am-C57BL/6 mouse, male	90 days	5	0, 2.8, 5.2, 8.7
Thompson et al. (2012b)	F344 Rat, female	7 days	5	0, 0.015, 0.21, 2.9, 7.2, 20.5
		90 days	10	
Thompson et al. (2011)	B6C3F1 mouse, female	7 days	5	0, 0.024, 0.32, 1.1, 4.6, 11.6, 31.1
		90 days	10	

[a]Note: In the synthesis, male and female doses were rounded to the same values for simplicity.

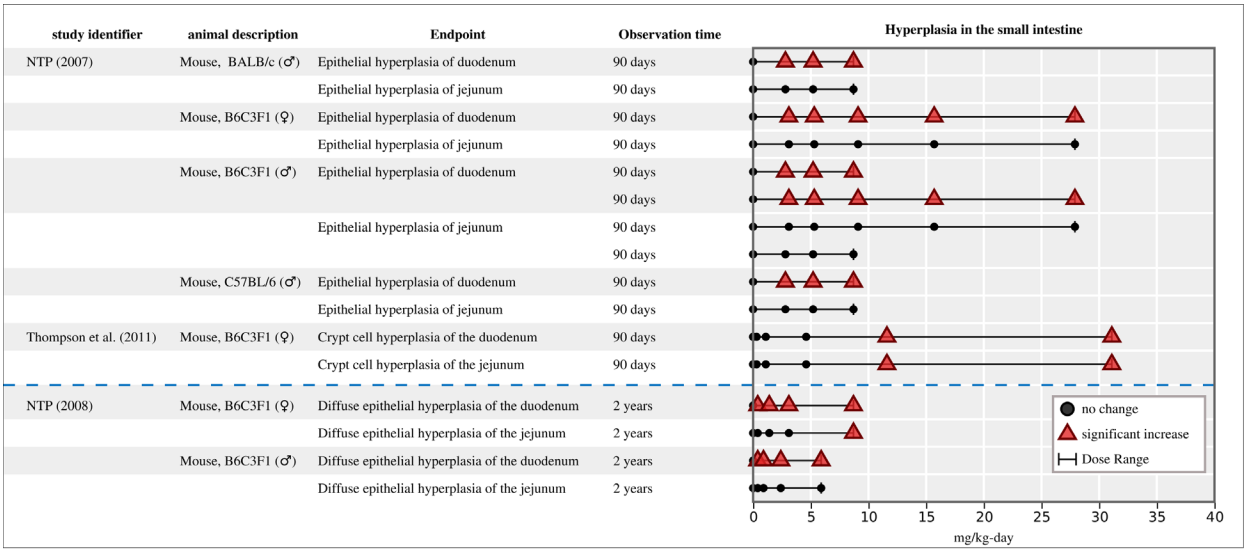

Figure 3-12. Diffuse epithelial hyperplasia in Cr(VI) treated mice in *high* confidence studies. Note: NTP (2008, 2007) did not present quantitative no-effect data. However, the dose levels and ranges for the exposure groups without effects are displayed here for comparative purposes. Click to see interactive data graphic.

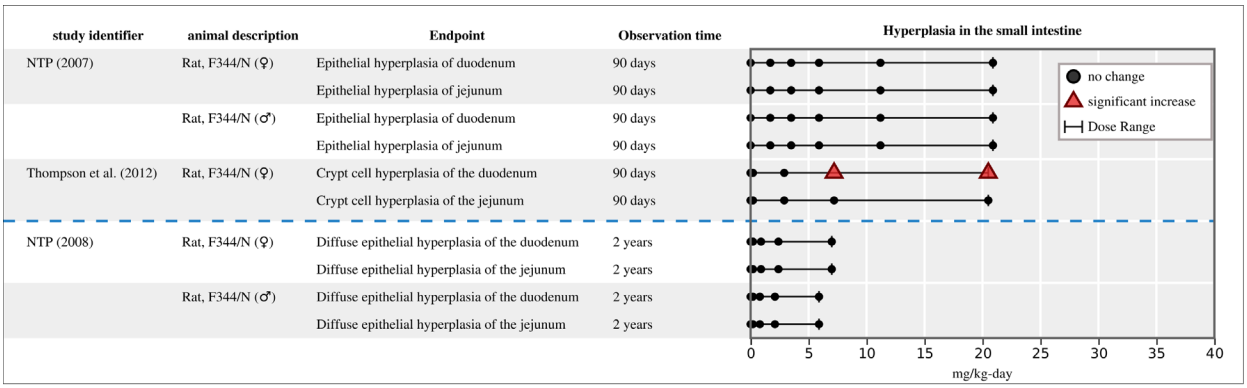

Figure 3-13. Diffuse epithelial hyperplasia in Cr(VI) treated rats in *high* confidence studies. Note: NTP (**2008**, **2007**) did not present quantitative no-effect data. However, the dose levels and ranges for the exposure groups without effects are displayed here for comparative purposes. Click to see interactive data graphic.

In subchronically exposed B6C3F1 mice, statistically significant elevated incidences of minimal to mild[25] diffuse duodenal epithelial cell hyperplasia were observed in both males and females at all doses (≥3 mg Cr(VI)/kg-d, incidence increasing with dose) (NTP, 2007). In a companion subchronic strain comparison study, statistically significant increases in the incidence of diffuse epithelial hyperplasia in the duodenum were also observed across all three strains of male mice tested (i.e., B6C3F1, BALB/c, and *am3*C57BL/6) (NTP, 2007). A separate subchronic study also showed a significant increase in duodenal hyperplasia in B6C3F1 mice at doses ≥11.6 mg Cr(VI)/kg-d (Thompson et al., 2011). This study did not show increasing incidence with dose, but the lowest dose level at which the epithelial hyperplasia was observed in Thompson et al. (2011) (~12 mg Cr(VI)/kg-d) was about 4x higher than for NTP (2007) (~3 mg Cr(VI)/kg-d), and resulted in dose-dependent apoptosis (which was statistically significant at the highest dose of 31.1 mg Cr(VI)/kg-d), which likely degenerated the duodenal tissue. The subchronic results of hyperplasia in the duodenum were consistent with a 2-year study that showed statistically significant elevated incidences of minimal to mild diffuse epithelial cell hyperplasia in the duodenum of the same severity but at lower doses (≥0.3 mg Cr(VI)/kg-d, incidence increasing with dose with the exception of the high dose males that had a slightly lower incidence than the second highest dose group) (NTP, 2008). In the jejunum, there were no significantly elevated increases in epithelial cell hyperplasia in either sex of B6C3F1 mice in a subchronic study at doses up to 28 mg Cr(VI)/kg-d (NTP, 2007), but in a second subchronic study, female mice of the same strain showed statistically significant elevated epithelial cell hyperplasia in the jejunum at doses ≥11.6 mg Cr(VI)/kg-d (Thompson et al., 2011). In the 2-year mouse study, this effect was observed in the jejunum of female mice at the highest dose (8.89 mg Cr(VI)/kg-d) (NTP, 2008). Together, these results show a

[25]According to NTP severity grading: 1=minimal, 2=mild, 3=moderate, 4=marked.

consistent pattern of minimal to mild diffuse epithelial hyperplasia in mice, which was present in subchronic studies at higher doses compared to the chronic study.

In subchronic and chronic NTP studies in F344 rats, increased diffuse epithelial hyperplasia was not observed in the small intestine (NTP, 2008, 2007). In contrast, a statistically significant increase in these lesions was observed following ≥7.2 mg Cr(VI)/kg-d exposures for 7 and 90 days in female F344 rats in a study by a separate group (Thompson et al., 2012b). The differences in the presence or absence of these lesions in F344 rats across studies is unknown, but this may have been affected by differences in water intake between the two study groups, leading to higher exposures to the rats in the the Thompson et al. (2012b) study. At the administered Cr(VI) concentrations, which were nearly equivalent between the studies, the mg/kg-d doses in the NTP subchronic bioassay (NTP, 2007) and the time weighted average doses from weeks 1–13 in the NTP chronic bioassay (NTP, 2008) were approximately twofold lower than the mg/kg-d doses in Thompson et al. (2012b)). In addition, Thompson et al. (2012b) noted that the animal vendor sources for the F344 rats were different between groups (NTP used animals from Taconic Farms, Inc. (NTP, 2008, 2007) and Thompson et al. (2012b) used animals from Charles River Laboratories International, Inc.), although the mice used by each group were also procured from these two different sources, respectively.

In the rat glandular stomach, there were also observations of epithelial hyperplasia along with several other lesion types in a subchronic but not chronic studies. These lesions were not observed in a subchronic study of three different strains of mice, nor in a chronic mouse study. Statistically significant increased incidences of epithelial hyperplasia, squamous metaplasia, and ulcers in the glandular stomach were reported in male and female F344 rats exposed to 21 mg Cr(VI)/kg-d (the highest dose) in the 13-week NTP study (NTP, 2007). No statistically significant increased incidences of glandular stomach or forestomach lesions were reported in the 2-year studies of F344 rats and B6C3F1 mice (NTP, 2008), or in the NTP (2007) 13-week studies of B6C3F1, BALB/c, or *am3*-C57BL/6 mice. Neither of the Thompson et al. (2012b; 2011) 13-week studies conducted histologic examinations of the forestomach or glandular stomach of mice or rats. The inconsistency between subchronic and chronic study results in rats is likely attributable to dose selection; in the 13-week study, stomach lesions occurred at an exposure that was threefold higher than the highest dose administered in the 2-year chronic assay.

Degenerative changes to the cells lining the GI tract can manifest as necrosis, apoptosis, and subsequent villous stunting, resulting in crypt abscess and ulceration (Betton, 2013). The NTP subchronic bioassay reported that the duodenal villi of B6C3F1 mice were short, thick, and blunted, with cytoplasmic vacuolization in the epithelial cells lining the villi tips at doses up to 27.9 mg Cr(VI)/kg-d (results were not presented quantitatively) (NTP, 2007). Consistent with these results, the NTP 2-year bioassay qualitatively reported degenerative effects in mouse duodenal villi (described as short, broad, and blunt) at doses up 8.89 mg Cr(VI)/kg-d. These effects were not reported in F344 rats at doses up to 21 or 7.13 mg Cr(VI)/kg-d after subchronic exposure or

chronic exposure respectively (NTP, 2008, 2007). GI tissue atrophy and apoptosis were not reported in the NTP bioassays in either species (NTP, 2008, 2007). Although cytoplasmic vacuolization, when irreversible, can be considered a marker of cell death due to cytoprotective autophagy in response to stress (Shubin et al., 2016), the vacuolization observed in epithelial cells at the tips of villi in mice in the subchronic study was not interpreted by NTP to be indicative of atrophy or apoptosis and was not observed in the 2-year bioassay (NTP, 2008, 2007). There was an increased incidence of minimal to mild salivary gland atrophy in female rats after two years at the two highest doses (the effect at the highest dose lacked statistical significance), although this effect is of unknown biological significance.

Thompson et al. (2011) reported degenerative changes in the intestines of female B6C3F1 mice after subchronic exposure including statistically significant atrophy in villi of the duodena and jejuna (31.1 mg Cr(VI)/kg-d, highest dose), apoptosis in the duodenal villi (31.1 mg Cr(VI)/kg-d), and cytoplasmic vacuolization in the duodena and jejuna (≥4.6 mg Cr(VI)/kg-d) (Figure 3-14). These results are generally consistent with the descriptive observations reported by NTP in mice after subchronic and chronic exposure. While the subchronic NTP study did not report identical histopathological findings, it stated that "the epithelial cells lining the tips of the villi of many of the exposed mice were swollen and had vacuolated cytoplasm. Collectively, these duodenal lesions suggest regenerative hyperplasia secondary to previous epithelial cell damage or degeneration" (NTP, 2007). The subchronic study in female F344 rats by Thompson et al. (2012b) also reported apoptosis of the duodenal villi at the two highest doses (7.2 and 20.5 mg Cr(VI)/kg-d), but no atrophy or vacuolization (Figure 3-14).

Two follow-up publications using the same experimental subchronic dataset in female B6C3F1 mice (Thompson et al., 2011) reported increases in some markers of duodenal villus cytotoxicity described as karyorrhectic nuclei, desquamation, villous blunting, and disruption of cellular architecture in the duodenal villi at doses ≥4.6 mg Cr(VI)/kg-day (Thompson et al., 2015a; O'Brien et al., 2013). It should be noted that O'Brien et al. (2013) only evaluated one animal in the next-lowest dose group (1.1 mg Cr(VI)/kg-day) for desquamation and disruption of cellular arrangement. In the crypt compartment, although increases in crypt length, area, and number of crypt enterocytes were reported, there were no statistically significant or dose-responsive changes in mitotic or apoptotic indices (Thompson et al., 2015a; O'Brien et al., 2013). Observations after 7-day exposures reported by this group (considered supporting evidence due to the short duration) include duodenal hyperplasia, villous atrophy, and cytoplasmic vacuolization, but again with no changes in crypt apoptosis indices, mitotic activity, or increases in karyorrhectic nuclei in the crypt compartment (Thompson et al., 2015b; Thompson et al., 2011). The authors attribute this discrepancy to either the 24-hour period without Cr(VI) exposure prior to sacrifice and/or to the sudden increase in the number of crypt enterocytes that then migrated toward the villus and became post-mitotic in that 24-hour period, apparently as mitotic figures were being measured (Thompson et al., 2015a; O'Brien et al., 2013).

While NTP (2008) noted short, broad, and blunt duodenal villi in mice, they did not report observing duodenal villus atrophy. In a second review of the NTP 2-year bioassay mouse histopathology slides by Cullen et al. (2015), these authors reported villus atrophy and blunting in all mice in the highest dose group. Cullen et al. (2015) also only observed cytoplasmic vacuolization in males; NTP made a general statement that vacuolization was observed in the tips of the villi without presenting incidence or details. While there were some descriptive reporting differences across studies for nonneoplastic histopathological lesions, an independent expert pathology review (Francke and Mog, 2021) of the diagnostic criteria used by these reports (Cullen et al., 2015; Thompson et al., 2015a; NTP, 2008, 2007) confirmed there was no meaningful difference or improvement when comparing the five histological diagnoses applied by this second review (ACC, 2015; Cullen et al., 2015) to those used by NTP. In fact, NTP addressed four of the five diagnostic terms used by Cullen et al. (2015) (i.e., histiocytic cellular infiltrates, atrophy/blunting, enterocyte vacuolation, and epithelial hyperplasia), with the exception of single-cell necrosis (i.e., apoptosis). Thus, the "short, broad, blunt" duodenal villi of exposed mice reported by NTP (2008) are analogous to the Cullen et al. (2015) report of "atrophy/blunting" of the villus.

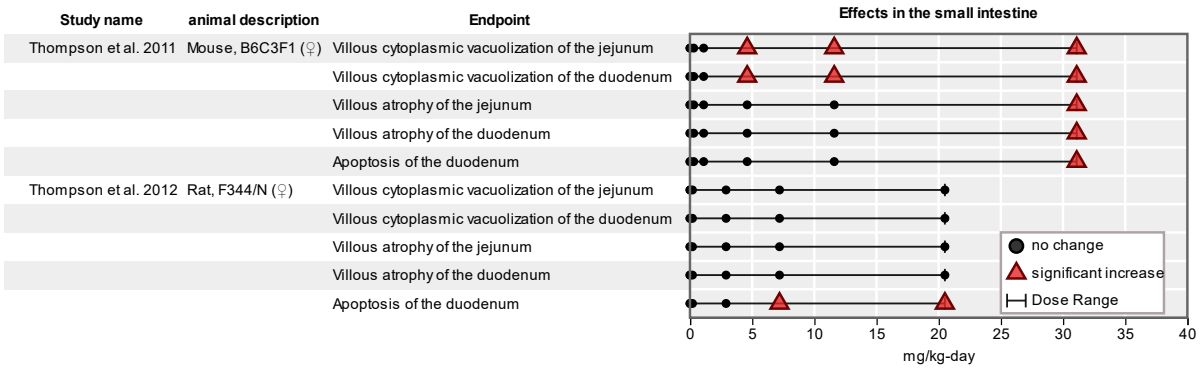

Figure 3-14. Cr(VI)-induced degenerative changes in the small intestines of mice and rats in high confidence studies. Click here to see interactive graphic.

Increased infiltration of histiocytes (macrophage immune cells) in the duodenum and jejunum was consistently observed in both sexes of rats and mice orally exposed both chronically and subchronically to Cr(VI) (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007). NTP (2008) indicated that the biological significance of the histiocytic infiltration is not known, but surmised that the infiltration of macrophages may reflect phagocytosis of an insoluble chromium precipitate. It should be noted that while macrophage accumulation may be associated with inflammation, NTP did not report chronic inflammation in the GI tract, or the influx of other inflammatory cells associated with the histiocytic infiltration in the small intestine (NTP, 2008, 2007).

In summary, diffuse epithelial hyperplasia of the small intestine was consistently observed in the three *high* confidence studies in mice, occurring at higher doses in the subchronic studies

compared to the chronic study, with similar severity across studies. Diffuse epithelial hyperplasia was also observed in the rat small intestine, but these findings were inconsistent between the two reporting groups. Similar degenerative changes in the duodenal villi were consistently observed across studies, and although the description of these effects varied, the results were essentially the same. Histiocytic infiltration was also consistently observed, though this effect was interpreted by the report authors to be of unknown biological significance (NTP, 2008) and is likely not adverse on its own.

3.2.2.3. *Mechanistic Evidence*

The screening and identification of mechanistic studies for evidence relevant to Cr(VI)-induced oxidative stress, cell proliferation and cell death in the GI tract prioritized both oral exposure studies in animals and studies via all routes in animals if results were presented for GI tissues, as well as in vitro studies in human cells derived from GI tissues (primary and immortalized); this prioritization strategy and a summary of the studies can be found in Appendix C.2.2.2. No human oral exposure studies or human studies of cytotoxicity or cell proliferation specific to the GI tract were identified. Because mechanistic evidence from studies of non-malignant toxic effects specific to the GI tract (in vivo or in vitro) following the ingestion of Cr(VI) is also relevant to cancer of the GI tract, a summary of this evidence is presented in Section 3.2.3.3. The evidence supports a consistent, coherent, and biologically plausible role for oxidative stress, cytotoxicity, and cell proliferation induced by Cr(VI) exposure in both the nonneoplastic toxicity and carcinogenic effects of Cr(VI) in the GI tract.

Three in vivo studies were identified that reported biomarkers of oxidative stress in GI tissues after oral exposure (Thompson et al., 2012b; Thompson et al., 2011; De Flora et al., 2008). In addition, a gavage study (Sengupta et al., 1990) reported various oxidative stress parameters in GI tissue after administration of potassium dichromate at doses of 1500 mg/kg-bw for three days and 300 mg/kg-bw for 30 days. However, the inclusion of doses that are higher than the LD50 (130 mg/kg) for rats (Thermo Fisher, 2009) is considered a limitation for interpreting the results of this study.

In female B6C3F1 mouse GI tract tissues, the reduced-to-oxidized glutathione ratio (GSH/GSSG), which is considered a biomarker of redox status, showed statistically significant, dose-dependent decreases in the oral and duodenal epithelium in mice exposed to Cr(VI) in drinking water (≥11.6 mg Cr(VI)/kg-d and ≥4.6 mg Cr(VI)/kg-d, respectively) after 7 days of exposure, indicating an increase in oxidative stress, with no correlated change in the GSH/GSSG ratio in plasma (Thompson et al., 2011). After 90 days, there was still a significant decrease in the GSH/GSSG ratio in the small intestinal epithelia of the duodenum (up to a 38.5% decrease at the top dose) and jejunum (up to a 52% decrease at the top dose), but not in the ileum, at concentrations ≥1.1 mg Cr(VI)/kg-d and decreases in plasma at higher concentrations (≥11.6 mg Cr(VI)/kg-d), but no decreases were detected in the oral mucosa despite a measurable chromium concentration in these tissues. While GSH/GSSG ratio measurement is a generally accepted indicator of oxidative

stress, ascorbate is the preferred in vivo reductant accounting for 90% of Cr(VI) oxidative metabolism (described in detail in Section 3.1.1.). Although the expected primary oxidative pathway is not captured in these experiments, the decreased GSH/GSSG ratio with increasing dose implies some level of Cr(VI)-induced oxidative stress was occurring in the duodenum. However, protein carbonyls, an indicator of protein oxidation, were only slightly elevated in the duodenum after 90 days (Thompson et al., 2011), possibly indicating that the ROS mediated damage is being preferentially directed at nucleic acids rather than proteins, although the reason for this preference is not known.

This study also did not observe increases in 8-OHdG DNA adducts in the oral cavity or duodenal tissue of mice (Thompson et al., 2011). The absence of oxidatively induced 8-OHdG adducts in mouse GI tissues is consistent with a study by De Flora et al. (2008), which found no increase in these lesions in the forestomach, glandular stomach, or duodenum after female SKH-1 mice were exposed for 9 months via drinking water at concentrations of 1.20 and 4.82 mg Cr(VI)/kg-d. The reason for the lack of oxidative DNA lesions associated with the oxidative stress in these studies is not known.

In female F344/N rats, Thompson et al. (2012b) reported no statistically significant changes in GSH/GSSG ratios in either the oral cavity or the small intestine of female rats after 7 days of Cr(VI) exposure to concentrations 0.1–180 mg/L Cr(VI), with the exception of decreases in the jejunum at the high concentration of 180 mg/L Cr(VI) and a decrease at 0.1 mg/L Cr(VI) in the oral mucosa. After 90 days, statistically significant and dose-dependent reductions in the GSH/GSSG ratio in the oral mucosa and jejunum were observed at concentrations ≥20 mg/L Cr(VI) (Thompson et al., 2012b). These results are in partial contrast to experiments in mice from the same research group (described above), which showed decreases in GSH/GSSG ratio in the duodenum but not the oral mucosa at 90 days despite mice having measurable total chromium concentrations in the oral cavity (Thompson et al., 2011). The plasma GSH/GSSG ratio was also decreased at concentrations ≥60 mg/L Cr(VI). No changes in the GSH/GSSG ratio were observed in the duodenum at 90 days, and there were no changes in 8-isoprostane, a marker of lipid peroxidation, in the oral mucosa or duodenum.

A large body of evidence from cells exposed in vitro demonstrates the increases in oxidative damage induced by Cr(VI), where ROS levels, lipid and protein oxidation, and decreased levels of antioxidant enzymes correlate with DNA damage that is increased in test systems with disabled DNA excision repair processes or abrogated with antioxidant pretreatment (Appendix Section C.3.6). This includes studies performed with human colon and gastric cancer cell lines to study oxidatively induced DNA damage and cytotoxicity. In vitro, it appears that Cr(VI) exposure can result in oxidative stress with minimal or no cytotoxicity, as shown in human colorectal adenocarcinoma Caco-2 cells (Thompson et al., 2012a). Thompson et al. (2012a) measured both 8-OHdG adducts and levels of phosphorylated histone H2AX (γH2AX), a marker of DNA double-strand breaks that could arise from various sources including ROS and/or direct chemical

interactions. After 24 hours, cytotoxic concentrations of Cr(VI) increased 8-OHdG and γH2AX levels, while non-cytotoxic concentrations only elevated 8-OHdG, suggesting that oxidative stress could be a mechanism for DNA damage other than double-strand breaks at lower concentrations in in vitro test systems. Notably, these results conflict with the in vivo study results following subchronic Cr(VI) exposure in drinking water presented above, which consistently showed no changes in 8-OHdG.

In the same study, Thompson et al. (2012a) reported that differentiated Caco-2 cells were more resistant to cytotoxicity than undifferentiated cells. There were no reported changes in immunofluorescence staining of differentiated Caco-2 cells for p53 or annexin-V (apoptosis markers) or LCB3 (an autophagy indicator). There was, however, a dose-dependent translocation of ATF6 to the nucleus in differentiated cells, which is an indicator of endoplasmic reticulum stress and supports in vivo toxicogenomic data indicating this response in duodenal tissue (Kopec et al., 2012b; Thompson et al., 2012a). A study by a separate group with the human gastric cancer cell line SGC-7901 showed that Cr(VI) treatment in cells modified by knockdown of URI (a transcription factor and oncogene) enhanced ROS production and cell death compared to control cells treated with Cr(VI) (Luo et al., 2016). This suggests URI may have a role in suppressing Cr(VI)-induced oxidative stress and apoptosis.

Tissue injury induced by cytotoxicity and oxidative stress in the GI tract may lead to necrosis and/or regenerative proliferation, evidenced by the histological degenerative changes in the small intestinal villi of mice exposed to Cr(VI) up to 2 years, as well as in the small intestine and glandular stomach of rats exposed for 3 months. While ultimately only mice developed intestinal tumors, the observations of hyperplasia, metaplasia, and ulcer in the stomach and villous wounding in the intestine of rats are similarly demonstrative that Cr(VI) may cause GI toxicity through tissue injury. As described in the synthesis of animal evidence, observations indicative of degenerative changes in the mouse small intestine were reported across studies and suggest a regenerative response to epithelial cell injury (Thompson et al., 2011; NTP, 2008, 2007). These Cr(VI)-specific effects in the small intestine are supported by X-ray fluorescence data showing ingested Cr concentrates in the duodenal villi of mice (Thompson et al., 2015b; Thompson et al., 2015a; O'Brien et al., 2013; Thompson et al., 2011). In the duodenum, diffuse hyperplasia was observed at all doses after both subchronic (≥3 mg Cr(VI)/kg-d) and chronic (≥0.3 mg Cr(VI)/kg-d) exposure, and focal hyperplasia was observed after chronic exposure at doses ≥2.4 mg Cr(VI)/kg-day.

Tissue injury in the mouse duodenal villi may lead to a compensatory proliferative response in the crypt compartment and hyperplasia observed in the intestinal mucosa as observed by dose-dependent crypt enterocyte proliferation (Thompson et al., 2015b; O'Brien et al., 2013), although the relationship between this measure of increased cell proliferation after a 7-day exposure and the observations of villous hyperplasia after 3 months or 2 years of exposure are unclear. These investigators observed increased numbers of crypt enterocytes but did not detect a treatment-

related increase in mitotic indices in these crypts, which would appear to be inconsistent with regenerative crypt hyperplasia (Thompson et al., 2015b; O'Brien et al., 2013).

Perturbations in cell signaling pathways that enhance cellular proliferation may contribute to the hyperplastic effects observed in the small intestine of B6C3F1 mice. Gene expression profiling studies of the tissues collected in the subchronic drinking water exposure study by Thompson et al. (2011) found that Ki-67 expression, a protein associated with cell proliferation used to label proliferative intestinal crypt compartment cells (Li et al., 2015a; Basak et al., 2014), was increased within the duodenal mucosa in mice at the two highest doses (11.6 and 31 mg/kg-d Cr(VI)) by day 91 (with dose-dependent increases at ≥4.6 mg/kg-day Cr(VI) at day 8) (Rager et al., 2017; Kopec et al., 2012a). A separate group reported that after 60 days of exposure to Cr(VI) in drinking water, the c-Myc oncogene showed a dose-dependent increase in the stomach (gene expression and protein levels ≥3.5 mg/kg-day Cr(VI)) and colon (gene expression ≥1.7 mg/kg-day and protein levels ≥5.2 mg/kg-day Cr(VI)) of male Wistar rats (Tsao et al., 2011), consistent with the promotion of cell cycle progression and cell proliferation. The same study also reported a decrease in the expression of RKIP (Raf kinase inhibitor protein; ≥5.2 mg/kg-day Cr(VI)), which is thought to negatively regulate MAPK (mitogen activated protein kinase) signaling involved in cellular proliferation (Vandamme et al., 2014). The gene expression and protein levels of tumor suppressor and cell cycle regulator p53 were also downregulated in the stomach (gene expression ≥3.5 mg/kg-day and protein levels ≥1.7 mg/kg-day Cr(VI)) and colon (gene expression and protein levels ≥5.2 mg/kg-day Cr(VI)) (Tsao et al., 2011). Consistent with these studies, toxicogenomic analyses of GI tissues in Cr(VI)-treated animals have identified differentially expressed genes (DEGs) associated with activation of c-Myc, MAPK, and a variety of additional pathways associated with cell cycle, proliferation, and apoptosis. A summary of gene expression changes and toxicogenomic results most pertinent to both noncancer and cancer GI effects can be found in Appendix C.3.3 and C.3.4, respectively, and is discussed in the context of cancer MOA in Section 3.2.3.

Although the molecular pathways leading to the cytotoxic effects of Cr(VI) in the GI tract following oral exposures are not clear, it is likely to involve chronic oxidative stress known to occur across multiple tissues following Cr(VI) exposures (see Section 3.2.3.3), though there are also indications of oxidative stress occurring in the absence of cytotoxicity. The data from studies of Cr(VI) provide consistent support for oxidative stress as a mechanism of Cr(VI) toxicity in the lung (Section 3.2.1), liver (Section 3.2.4), male and female reproductive organs (Sections 3.2.7 and 3.2.8, respectively), and fetal development (Section 3.2.9), though in vivo results specific to the GI tract are mixed (Thompson et al., 2013). Proliferative cell signaling pathways show upregulation in the GI tract that is generally consistent with the pathological evidence of tissue regeneration in the mouse small intestine, though it cannot be conclusively determined whether these dose-dependent gene expression and protein level changes are associated with compensatory cell proliferation following cytotoxicity or are induced by Cr(VI) exposure via another pathway.

3.2.2.4. *Integration of Evidence*

Overall, the currently available **evidence indicates** that oral exposure to Cr(VI) likely causes GI tract toxicity in humans under relevant exposure circumstances. This evidence is summarized in Table 3-10. This conclusion is based on *robust* studies in rodents that found Cr(VI) causes nonneoplastic effects in the GI tract at doses ≥0.3 mg/kg-d Cr(VI) (NTP, 2008). These effects include dose-responsive diffuse epithelial hyperplasia in mice after both subchronic and chronic exposure at all doses, and degenerative changes in the rat and mouse intestine. Human evidence for nonneoplastic effects in the GI tract was *indeterminate* due to a lack of studies of chronic, nonneoplastic GI effects in humans. The ATSDR Toxicological Profile (ATSDR, 2012) described multiple case reports of Cr(VI) induced GI toxicity or deaths among adults and children but none included an appropriate comparison group.

The animal toxicological database provides *robust* evidence that Cr(VI) is toxic to the GI tract. The primary nonneoplastic effects associated with both chronic and subchronic oral exposure to Cr(VI) in the GI tract are consistent and biologically coherent, and include epithelial cell hyperplasia, degenerative changes, and histiocytic cellular infiltration in the small intestine. Diffuse epithelial hyperplasia of the small intestine was predominant in mice across all studies, with incidence increasing with dose. NTP observed diffuse epithelial hyperplasia, which involved the entire small intestinal mucosa, in all exposed groups (≥0.3 mg/kg-d Cr(VI)) of males and females in both subchronic and chronic studies (NTP, 2008, 2007). The incidence rate was high (>26%) at the lowest dose. Other subchronic experiments, including a strain comparison study by NTP, also observed these lesions in mice (Thompson et al., 2011; NTP, 2007). The dose-response relationship for epithelial hyperplasia was stronger in the proximal small intestine (duodenum) than it was in the jejunum (see Figure 3-12), indicating the effects of Cr(VI) are diminished by a decrease in concentration as the chemical traverses the small intestine[26]. In addition to diffuse hyperplasia, there was a low, nonsignificant incidence of focal epithelial hyperplasia in the duodenum observed by NTP after 2 years in both male and female mice at the mid and high doses. These lesions are discussed further in Section 3.2.3.2 as they may be more indicative of a direct treatment-related preneoplastic response.

In rats, epithelial hyperplasia and villus atrophy/blunting were only reported in one subchronic study limited to females (≥7.2 mg and 31.1 mg/kg-d Cr(VI) respectively) (Thompson et al., 2012b). Histopathological discrepancies in the rat small intestine between these findings and the NTP (2008, 2007) studies are a source of uncertainty, but could involve differences in study variables such as those described by Thompson et al. (2012b) (e.g., different vendor sources, differences in water intake), or differences in analyses (i.e., comprehensive pathology reporting by NTP vs. hypothesis-driven MOA studies by Thompson et al. (Francke and Mog, 2021)). In the

[26]As Cr(VI) traverses the small intestine, the concentration of Cr(VI) in the lumen decreases due to 1) reduction of Cr(VI) to Cr(III), 2) uptake to the small intestine epithelium, 3) dilution by GI contents (including by ongoing intestinal secretions). See Section 3.1.

glandular stomach, a significantly increased incidence of nonneoplastic lesions was seen in male and female F344 rats exposed to the highest dose (21 mg/kg-d Cr(VI)) in the subchronic NTP study; this effect was not observed at any dose after two years (NTP, 2008, 2007). This is likely explained by differences in dosing, as the rat stomach lesions observed after 13 weeks occurred at an exposure threefold higher than the highest dose in the 2-year chronic assay.

Observations of histiocytic infiltration in the small intestine were consistent across studies, sexes, and species; however, this effect is of unknown biological significance. Histiocytic infiltration (to varying degrees) was also observed in the liver and the pancreatic and mesenteric lymph nodes (NTP, 2008, 2007). A plausible explanation for this effect is increased phagocytosis due to an insoluble precipitate of the test material. Cr(III), the reduced form of Cr(VI), is not a substrate for active transport through the cell membrane and would therefore enter cells through passive diffusion or phagocytosis (Witt et al., 2013). Therefore, the observed histiocytosis is most compatible with phagocytically active macrophages containing Cr(III). An alternative explanation could be that histiocytosis occurred as a result of chronic inflammation; however, neither pathology consistent with inflammation nor the presence of other inflammatory cells types were observed in rats or mice following drinking water exposures (NTP, 2008, 2007).

Together, these effects provide consistent, biologically coherent evidence of GI toxicity involving tissue wounding by the test substance leading to degenerative changes, regenerative proliferation and hyperplasia. The hyperplasia in the GI tract following oral exposures is considered to be representative of the constellation of histopathological observations that together result in a change in tissue function that is considered an adverse noncancer effect, independently from the significance of this lesion as a preneoplastic effect in the potential progression to cancer. Mechanistic evidence from in vitro and in vivo models provides additional support for GI tissue cytotoxicity and apoptosis occurring as a result of Cr(VI) exposure, as well as a proliferative response that may be directly associated with a Cr(VI)-induced stimulation of proliferative cell signaling pathways, an indirect consequence of compensatory cell proliferation following tissue injury, or a combination of both.

Table 3-10. Evidence profile table for effects in the GI tract other than cancer

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from studies of exposed humans (occupational multi-route)					⊕⊕⊙ The **evidence indicates** that Cr(VI) is likely to cause GI toxicity in humans. *Robust* evidence in rats and mice shows consistent findings of histopathological changes indicative of epithelial damage and changes in GI epithelial architecture following oral exposure. Although these effects are presumed to be relevant to humans, the lack of human evidence demonstrating that the changes observed in rodents would occur and progress in humans precludes a higher conclusion level (i.e., *evidence demonstrates*). Mechanistic findings in animals provide some evidence supportive of
No human studies met PECO criteria for nonneoplastic GI effects	For human evidence of cancer of the GI tract, see Section 3.2.3.2 and Table 3-24 (Evidence profile table for cancer of the GI tract).			⊙⊙⊙ *Indeterminate*	
Evidence from animal studies (oral)					
HISTOPATHOLOGICAL CHANGES **High confidence:** NTP (2008) NTP (2007) Thompson et al. (2012b) Thompson et al. (2011)	All studies examining effects in the small intestine are rated high confidence. Degenerative changes in intestinal villi and hyperplasia of the small intestine observed in male and female mice by NTP (2008, 2007), and in female mice and rats by Thompson et al. (2012b; 2011). Histiocytic cellular infiltration observed in the small intestine of male and female rats and mice in all studies and bioassays. Because these effects can also represent preneoplastic lesions that are part of the morphologic and biologic continuum leading to cancer (Boorman et al., 2003),	• Consistent findings in mice in four *high* confidence studies which contained multiple bioassays (both sexes and multiple strains of mice) • Coherence-biologically related findings across studies • Large magnitude of effects • Strong dose-response gradient • Mechanistic evidence (oxidative stress, cell proliferation) provides plausibility	• Inconsistent findings in rats (intestinal hyperplasia observed in two of four *high* confidence studies), although this inconsistency between species may be partly explained by pharmacokinetics	⊕⊕⊕ *Robust* Histopathological changes reported in high confidence studies (proliferative changes) observed across the animal evidence base database are coherent following chronic and/or subchronic oral exposures in rats and mice and suggest adverse effects of Cr(VI) on the GI tract (specifically, the small intestine), findings that are supported by mechanistic evidence of oxidative stress and cell proliferation.	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	additional discussions are provided in Section 3.2.3.2 (Gastrointestinal Tract Cancer) and Table 3-24.				oxidative stress in the GI tract as a potential mechanism for degenerative GI effects in multiple animal species. This mechanism is presumed relevant to humans. The **evidence is inadequate** to determine whether Cr(VI) inhalation exposure might be capable of causing noncancer GI effects. No noncancer GI effects were observed following inhalation. As described in Section 3.1, Cr(VI) can expose portal-of-entry tissues, and reduction of Cr(VI) in these tissues and red blood cells decreases uptake by other organ systems.
Mechanistic evidence					
Biological events or pathways	**Summary of key findings and interpretations**			**Judgments and rationale**	
Oxidative stress	*Interpretation:* Cr(VI) is a potent oxidizer that can produce reactive oxygen species and oxidative stress via intracellular intermediate species, leading to cytotoxicity in the GI tract following oral exposures. This supports evidence of degenerative lesions in the GI tract (see animal evidence, above). *Key findings:* • Decreased GSH/GSSG ratio in small intestinal epithelium at 8 and 90 days in mice and 90 days in rats, and in oral mucosa in mice at 8 days and rats at 90 days, although no 8-OHdG adducts or protein oxidation in any tissues (Thompson et al., 2011; De Flora et al., 2008) • In vitro evidence of increased oxidative stress in human colorectal adenocarcinoma Caco-2 cells, though this also occurred at concentrations that induced minimal or no cytotoxicity (Thompson et al., 2012a)			Biologically plausible mechanistic evidence supports involvement of oxidative stress in the histopathological findings of degenerative effects, although there are some inconsistencies in the animal findings in the GI tract following oral exposures. Evidence of increased cell proliferation in affected tissues is consistent with hyperplasia but cannot be conclusively associated with tissue regeneration following injury.	
Cell proliferation	*Interpretation:* Evidence of increased cell proliferation is consistent with the histopathological observations of hyperplasia in the mouse small intestine following oral exposure to Cr(VI) (see animal evidence, above), although these measures do not indicate the molecular stimuli for the proliferation and it is unknown whether they are indicative of regenerative proliferation. Increased cell proliferation has not been detected in the rat oral cavity. *Key findings:*				

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	• The cellular replication marker Ki-67 was increased in isolated duodenal mucosal cells from the small intestine of mice exposed to Cr(VI) via drinking water for 7 and 90 days (Rager et al., 2017; Kopec et al., 2012a) • Dose-dependent increases in the protein and gene expression of c-Myc, an oncogenic cell proliferation promoter, and downregulation of cell cycle regulator p53, in rat stomach and colon exposed to doses as low as 5 mg/kg-d Cr(VI) in drinking water for 60 days of exposure to Cr(VI) in drinking water (Tsao et al., 2011) • Toxicogenomic analyses of GI tissues in Cr(VI)-treated animals have identified differentially expressed genes (DEGs) associated with activation of c-Myc, MAPK, and a variety of additional pathways associated with cell cycle and proliferation (see Appendix C.3.4)				

3.2.3. Cancer

3.2.3.1. *Respiratory Tract Cancer*

In 1998, the EPA IRIS Toxicological Review of Hexavalent Chromium classified Cr(VI) as a "known human carcinogen by the inhalation route of exposure" based on consistent evidence that inhaled Cr(VI) causes lung cancer in humans and supporting evidence of carcinogenicity in animals. The same conclusion has since been reached by other authoritative federal and state health agencies and international organizations and the carcinogenicity of Cr(VI) is considered to be well established for inhalation exposures (TCEQ, 2014; IPCS, 2013; NIOSH, 2013b; IARC, 2012; CalEPA, 2011; NTP, 2011; OSHA, 2006). Thus, the current review of cancer by the inhalation route adopts the same EPA cancer descriptor for this route, *carcinogenic to humans*, and the analyses focus on data that may improve the quantitative exposure-response analysis conducted in EPA's 1998 IRIS assessment, as stated in the 2014 preliminary packages (U.S. EPA, 2014b, c) and the Systematic Review Protocol (Appendix A). An overview of the literature screening and study evaluation for exposure-response data is presented in Section 4.4.

3.2.3.2. *Gastrointestinal Tract Cancer*

Human Evidence via the Oral Route of Exposure

Study evaluation summary

Three studies analyzed stomach cancer risk in populations exposed to Cr(VI) in drinking water. Three additional studies were identified but excluded due to critically deficient ratings in at least one domain, and are not discussed further (Fryzek et al., 2001; Bick et al., 1996; Bednar and Kies, 1991). The three included, *low* confidence studies are ecological analyses of cancer mortality in residential populations with potential exposure to Cr(VI)-contaminated drinking water in China and Greece (Table 3-11).

Two of the studies were ecological analyses of cancer mortality in relation to groundwater contamination in the same exposed population in Liaoning Province, China (Kerger et al., 2009; Beaumont et al., 2008). The Beaumont et al. (2008) and Kerger et al. (2009) studies are reanalyses of Zhang and Li (1987), the original scientific report published in the Chinese Journal of Preventive Medicine. Another publication, Zhang and Li (1997), has been challenged for conflict-of-interest due to undisclosed funding[27]. Investigators compared cancer mortality rates (total between 1970–1978) between five contaminated regions identified along a groundwater plume of Cr(VI) and four presumed uncontaminated regions surrounding a ferrochromium production plant. The contaminated areas included five communities downgradient of the alloy plant along a dry riverbed where plant wastewater effluent from chromium smelting had been disposed since 1960. The

[27]Zhang and Li (1997) was retracted by the journal because "financial and intellectual input to the paper by outside parties was not disclosed" (Smith, 2008; Brandt-Rauf, 2006).

communities without contamination included the town adjacent to the alloy plant (TangHeZi) and three agricultural areas to the north, west and south. Another study with an ecological design, Linos et al. (2011), analyzed cancer mortality and Cr(VI) exposure via drinking water in Oinofita municipality, Greece, with data on residents from 1999–2009. Processed liquid industrial waste containing Cr(VI) was dumped into Asopos River starting around 1969, which was the source for drinking water in wells within the municipality from 1970−2009 (Linos et al., 2011).

The definition of Cr(VI) exposure in these studies was based on living in towns or areas proximate to contaminated rivers, which were the source of drinking water, and assumed consumption. Individual-level data on the source or amount of drinking water consumed was not collected. Sampling to measure Cr(VI) concentrations in drinking water was limited in terms of timespan as well as geographical coverage. In addition, only drinking water in the areas with suspected contamination was sampled; Cr(VI) concentrations were not measured in drinking water in areas considered to be unexposed, which could lead to unrecognized exposure and subsequent misclassification (Linos et al., 2011; Kerger et al., 2009; Beaumont et al., 2008). Based on data for Liaoning Province reported by the Jinzhou Health and Anti-epidemic Station in 1986, concentrations of Cr(VI) in drinking water (analytical methods were not available) in 1965, when the contamination was identified, ranged between 0.002−20.0 mg/L in villages along the plume that extended from the disposal site located near the chromium alloy plant (Kerger et al., 2009; Beaumont et al., 2008). Well water samples collected in Oinofita municipality between 2007–2010 ranged between 0.010–0.156 mg/L (Linos et al., 2011).

The studies of both populations were classified as *low* confidence, primarily due to limitations in the exposure assessment. In each study, exposure was defined at the population level; no individual-level exposure assignments were possible. Beaumont et al. (2008) and Kerger et al. (2009) assigned exposure status based on residence information in the death certificate. Residence at the time of death may not represent residence location – and thus inferred Cr(VI) exposure – at the critical time window for initiation and progression of cancer, although such misclassification of the exposure proxy is expected to be nondifferential. In addition, the duration of follow-up in both studies was not adequate to allow for the long latency of cancer development. These limitations are expected to result in bias in a direction toward a null association. Finally, age-adjusted site-specific cancer mortality by region for the study years in China was not available to the investigators and had to be estimated using other available data.

Table 3-11. Summary of human studies for Cr(VI) cancer of the GI tract and overall confidence classification. Click to see interactive data graphic for rating rationales.

Author (Year)	Location	Exposure Assessment	Study Design	Selection	Exposure	Outcome	Confounding	Analysis	Sensitivity	Self-reporting	Overall confidence
Beaumont et al. (2008)[a]	Liaoning Province, China	Communities downstream of a ferrochromium plant versus unexposed communities (assumed)	Semi-ecologic cancer mortality	D	D	D	D	A	D	A	Low
Kerger et al. (2009)[a]	Liaoning Province, China	Communities downstream of a ferrochromium plant versus unexposed communities (assumed)	Semi-ecologic cancer mortality	D	D	D	D	A	D	A	Low
Linos et al. (2011)	Oinofita, Greece	Residents of Oinofita, a contaminated region versus surrounding residents	Semi-ecologic cancer mortality	A	D	A	A	A	D	A	Low

G = good; A = adequate; D = deficient.
[a]Studies are reanalyses of Zhang and Li (1997; 1987).

Each of the three studies selected the referent, or unexposed population, as the larger area surrounding the exposed area (Linos et al., 2011; Kerger et al., 2009; Beaumont et al., 2008), and were not able to account for differing lifestyles, occupational histories, or background rates of cancer in the referent population that may influence cancer risk. Beaumont et al. (2008) compared cancer mortality in the contaminated villages to mortality in either the surrounding unexposed villages, or the entire Liaoning Province, with both comparison groups including the industrial city of TangHeZi. Larger populations, such as a province or state, have the advantage of providing relatively stable estimates, particularly for low-incident events such as site-specific cancers, but may obscure differences by demographic and other characteristics important for the study population. Kerger et al. (2009) compared cancer mortality in the chromium-exposed agricultural areas to the unexposed agricultural areas and to the unexposed city of TangHeZi separately to address potential residual confounding by demographic and socioeconomic factors. Mortality rates for stomach cancer in TangHeZi were lower than those in the unexposed agricultural areas. Although an analysis of gastric cancer rates in China in 1990–1992 showed lower mortality rates in urban areas (15.3 per 100,000) compared with rural areas (24.4 per 100,000), possibly in response to economic development and urbanization (e.g., sanitation, refrigeration) (Yang, 2006), this same study reported little difference between urban and rural rates in 1973–1975 (20.1 and 19.4 per 100,000 in urban and rural areas, respectively), the relevant time period with respect to the

Liaoning Province studies given the anticipated latency of cancer development and diagnosis following the onset of exposure. Therefore, while it is possible that demographic differences influenced the difference in mortality rates, another factor may have been statistical instability due to small population sizes.

Synthesis of human evidence

Results of the studies on Cr(VI) oral exposure and cancer are presented in Table 3-12. The analyses of stomach cancer in two exposed populations in Liaoning Province, China, and Oinofita, Greece, showed an association with Cr(VI), although effect estimates were imprecise. While the results of two reanalyses of Zhang and Li (1987) indicated an increased risk when comparing the exposed villages to the unexposed referent group, inclusion of the industrial city of TangHeZi in the referent group increased the magnitude of the relative risk, which became statistically significant (including TangHeZi, RR 1.82, 95% CI: 1.11, 2.91; excluding TangHeZi, RR 1.22, 95% CI: 0.74, 2.01) (Kerger et al., 2009; Beaumont et al., 2008). The mortality rate from stomach cancer was much lower in TangHeZi, the reason why inclusion of the city was influential. However, Beaumont et al. (2008) also used the mortality experience of the larger province as a referent and observed an elevated, statistically significant risk (SMR: 1.69, 95% CI: 1.12–2.44). Unfortunately, the number of deaths from stomach cancer was not reported for one of the villages with higher contamination levels, which makes it difficult to compare results between the two studies.

Table 3-12. Associations between drinking water exposures to Cr(VI) and cancer in *low* confidence epidemiology studies

Reference	Exposure	Cancer Deaths (N)	Relative Risk	Ratio Measure (95% CI) N
Linos et al. (2011) Oinofita, Greece	Cr(VI) in drinking water Mortality in exposed areas compared to surrounding area (assumed to be unexposed)	All cancers (118) Stomach (6)[a]	SMR (95% CI)	All cancers: 113.6 (94.1, 136.1) **Stomach: 120.9 (44.4, 263.2)**
Beaumont et al. (2008) Liaoning Province, China	Cr(VI) in drinking water Mortality in exposed communities compared to nearby regions (assumed to be unexposed) and to province as a whole	All cancer (262)[b] Stomach cancer (75)[b, c]	Rate ratio (95% CI)	*Compared to unexposed regions*: All cancers: 1.13 (0.86, 1.46) **Stomach: 1.82 (1.11, 2.91)** *Compared to larger province*: All cancers: 1.23 (0.97, 1.53) **Stomach: 1.69 (1.12, 2.44)**

Reference	Exposure	Cancer Deaths (N)	Relative Risk	Ratio Measure (95% CI) N
Kerger et al. (2009) Liaoning Province, China	Cr(VI) in drinking water Mortality in exposed communities (C) compared to (A) industrial town, or (B) three unexposed agricultural villages	All cancer (263)[b] Stomach cancer (89)[b, c]	Rate ratio (95% CI)	*C vs. B* All cancers: 1.10 (0.80, 1.51) **Stomach: 1.22 (0.74, 2.01)** *B vs. A* All cancers: 1.03 (0.77, 1.39) **Stomach: 1.70 (1.00, 2.89)** *C vs. A* All cancers: 1.14 (0.85, 1.52) **Stomach: 2.07 (1.25, 3.44)**

[a]Site-specific cancer risk presented for number of cases >5.
[b]Number deaths in the study villages were estimated as described by authors.
[c]Mortality rates were missing for stomach cancer in one contaminated village, Nuer River Village.

The studies of both of the populations exposed to Cr(VI) in drinking water reported increased SMRs when their mortality experience was compared to unexposed communities in the surrounding areas. These estimates were imprecise and changed in magnitude depending on the definition of the unexposed communities. The lack of individual estimates of exposure, the uncertain nature of the mortality data, and the potential impact of confounding by differences in SES between comparison groups make it difficult to draw any conclusions.

Human Evidence via the Inhalation Route of Exposure

EPA conducted a review and meta-analysis of GI cancer risk from studies of workers with occupational inhalation exposure to Cr(VI). Exposure via inhalation may pose an increased risk of cancer in the GI tract in occupationally exposed populations either as a result of systemic absorption and distribution, or via deposition in airways, mucociliary clearance, and swallowing of particles (Sedman et al., 2006). Numerous studies have evaluated the association between Cr(VI) exposure and cancers of the GI tract, including at least three recent meta-analyses (Deng et al., 2019; Suh et al., 2019; Welling et al., 2015) and two older meta-analyses (Gatto et al., 2010; Cole and Rodu, 2005) (Table 3-13). These meta-analyses varied in their scope and the specific research question under study. Among the more recent meta-analyses, the Welling et al. study (Welling et al., 2015) concluded that Cr(VI) exposure was associated with increased risks of stomach cancer, while Suh et al. (2019) had the opposite conclusion; the work by Deng et al. (2019), which considered additional cancer sites, concluded that there was no evidence for increased risk of death due to digestive system cancers overall, but that the findings for rectal cancer specifically were suggestive of increased risk, and the risk of oral cancer incidence (not mortality) was significantly increased. EPA performed an updated literature search to identify studies for inclusion in a new meta-analysis of Cr(VI) exposure in relation to GI tract cancers. The goal of the meta-analysis was to calculate summary effect estimates for persons with likely occupational exposure to Cr(VI) from an updated set of studies with similar design. Methods for the systematic review and meta-analysis are in Appendix C.3.1.

Table 3-13. Meta-analyses of GI tract cancers and Cr(VI) occupational exposure

Study	Outcome	Included	Excluded	Summary effect estimate and 95% confidence interval for specified cancer sites (number of included studies)
Cole and Rodu (2005)	Relative risk (RR) estimates for stomach cancer	Began with set of known relevant studies, then performed a literature search; included those published after 1950	'no usable data'; 'occupational settings with little or no chrome exposure'	Stomach (n = 32): 1.13 (1.03, 1.24)
Gatto et al. (2010)	Measures of effect or data available to calculate relative risk (RR) for GI tract cancers	Published after 1950; occupational exposure (inhalation or ingestion); exposure potential stated explicitly or from industry with recognized exposure potential: chromate production, stainless-steel welding, chrome pigment production, chrome plating, ferrochrome production		Esophagus (n = 15): 1.17 (0.90, 1.51) Stomach (n = 29): 1.09 (0.93, 1.28) Colon (n = 13): 0.89 (0.70, 1.12) Rectum (n = 20): 1.17 (0.98, 1.39)
Welling et al. (2015)	Relative risk (RR) estimates for stomach cancer	Chromate or chromium production and plating; leather work and tanning; Portland cement work; and stainless-steel production, welding, polishing and grinding	Occupations such as painting, general foundry work, construction and shoe (non-leather) manufacturing; Welding or metal plating studies that did not evaluate stainless-steel or chromium work; Studies involving work with asbestos cement	Stomach (n = 56): 1.27 (1.18, 1.38)
Deng et al. (2019)	Standardized mortality or incidence ratio (SMR or SIR) estimates for cancer of the digestive system	"the exposure factor was clear and exposure was to Cr(VI)" Chromate production, cement production, cement industry workers, aircraft manufacturing workers, chromium platers, tanners, welders, masons	Occupational exposure to materials other than Cr(VI), such as asbestos or nickel; professions such as shoemaking (non-leather) or general building work. Based on study quality evaluation using Newcastle-Ottawa scale, excluded studies with ratings <6	Esophagus (n = 14): 0.88 (0.73, 1.05) Stomach (n = 33): 0.93 (0.78, 1.09) Colon (n = 12): 1.06 (0.93, 1.21) Rectum (n = 23): 1.14 (0.98, 1.33)

Study	Outcome	Included	Excluded	Summary effect estimate and 95% confidence interval for specified cancer sites (number of included studies)
Suh et al. (2019)	Stomach cancer morbidity and/or mortality	Chromate production, stainless-steel welding, chrome pigment production, chrome plating/ electroplating, ferrochrome production industries, Leather tanners (if indicate exposure to Cr(VI) or process such as "two bath" process), Cement workers (if involved cement production); Other occupations if Cr(VI) exposure indicated by authors	PMR studies, Registry studies where 'Specifications of Cr(VI) exposures are not indicated by the authors"—includes studies such as Andersen et al. (1999) and Pukkala et al. (2009). Based on study quality evaluations using NTP OHAT Risk of Bias Rating Tool for Human and Animal Studies, tiered studies and excluded tier 3.	Stomach (n = 44): 1.08 (0.96, 1.21)

Occupational studies that analyzed cancer risks related to Cr(VI) exposure were identified as part of the overall assessment search strategy process described in the Cr(VI) Protocol (U.S. EPA, 2019). This search strategy, which was conditioned on terms for Cr(VI), identified 35 potentially relevant citations. Since these searches only identified references that mentioned chromium or related terms in the title or abstract, an additional search strategy was developed to identify studies of occupational groups with likely exposure to Cr(VI). The search terms and literature identification results are found in Appendix C.3.1. In total, 35 references from the previous literature searches for the assessment, 93 references from the subsequent occupationally-focused search for the meta-analysis, and 20 references identified by looking through the reference lists in the three most recent meta-analyses were included in this review. Of these, 21 studies were not included because they were earlier follow-ups with more recent reports available, the cohorts were not exposed to Cr(VI), or they did not contain results for site-specific GI tract cancers.

A comparison of the studies included in the three most recent meta-analyses and this analysis with a rationale for decisions to exclude is in the appendix (Table C-52, Section C.3.1). The studies included in each meta-analysis comprised a partially overlapping set of studies reflecting the various time periods used for the literature searches, the inclusion criteria, and the results of the evaluations of study "quality" used in the studies. The meta-analyses focused on the studies considered to be *medium* or *high* overall confidence for which EPA had greater certainty in the exposure assessment for Cr(VI) and minimal concern for other sources of bias. In this analysis, the primary reason for considering a study to be of low confidence was that exposure to Cr(VI) in the population was too uncertain.

The studies included in EPA's meta-analysis reported a variety of effect estimates, including standardized incidence or mortality ratios, standardized risk ratios, odds ratios, and proportionate mortality ratios. Studies that calculated proportionate mortality ratios were not included. In some instances, multiple risk estimates were reported—for example, for men or women separately, for exposure or occupational subgroups, or by latency period. A priori, risk estimates were preferred if they (1) were adjusted for potential confounders including age, sex, time period, and geographic region; (2) were estimated for the longest latency period; (3) were from the most recent follow-up of a specific study cohort; (4) were estimated for the most highly exposed subgroup of the study population. When reviewing the studies captured by the literature search and evaluation of the studies, there were some cancer sites or groupings that were difficult to reconcile across studies due to differences in ICD codes included, for example, or changes in coding practices and diagnostic naming conventions over time and across geographical sites. Consequently, it was hard to determine whether the same cancer sites were contained within some of the groupings. Further, in some cases the number of studies for a given cancer site was small enough (and heterogenous enough) that a meta-analysis seemed unlikely to yield useful information. Consequently, a quantitative meta-analysis was performed to derive summary risk estimates for a subset of GI tract cancers by site: esophagus, stomach, colon, and rectum. For each of these four sites, there was a larger number of studies to include in a summary effect estimate, and these studies used relatively consistent definitions for these specific cancer sites.

Separate meta-analyses were performed to obtain summary estimates from studies reporting odds ratios (stomach cancer, esophageal cancer), and from studies reporting SMR, SIR, or SRR estimates (all four sites). All analyses were performed using the 'metafor' package in R (Viechtbauer, 2010), with a random effects model. This package was also used to generate forest plots (see Figures 3-15 to 3-21). The potential for publication bias was evaluated using the Egger's test (Egger et al., 1997) for funnel plot asymmetry. The I^2 statistic value for each study is used to represent the percentage of variation across studies that is due to heterogeneity rather than chance.

As shown in Table 3-14, the summary effect estimates showed small increases in risk for each cancer site associated with Cr(VI) exposure, although only the estimate for rectal cancer was statistically significant. There were few studies reporting odds ratios, but in each case (esophagus and stomach), summary effect estimates based on these studies were somewhat higher compared with summary estimates based on other relative risk measures (although neither odds ratio-based estimate was statistically significant). There was no evidence of funnel plot asymmetry based on Egger's regression test, indicating that publication bias was not likely to be present.

Summary effect estimates were also derived for each cancer site, stratified by occupational grouping (see Appendix Table C-45). This separation by occupational grouping did show some expected patterns for colon cancer risk estimates in that the occupations with a higher certainty of exposure to Cr(VI) (i.e., ferrochromium, chromate production, stainless-steel workers, chromium

pigment exposed workers) showed higher summary effect estimates. However, there remained inconsistencies among the studies overall, and the results for cancer of the rectum did not show a similar pattern of risk. The results of these more detailed analyses are discussed in Appendix C.3.1.3.

Table 3-14. Summary effect estimates from random effects meta-analysis, by cancer site and type of effect estimate

Cancer Site	Effect Estimate Type	Number of individual effect estimates	Summary effect estimate (95% confidence interval)	p-value for funnel plot asymmetry
Esophagus	Odds Ratio	2	1.43 (0.19, 11.09)	Not computed
	Relative Risk (SMR, SIR, or SRR)	21	1.08 (0.92, 1.37)[a]	0.33
Stomach	Odds Ratio	4	1.38 (0.77, 2.49)	0.79
	Relative Risk (SMR, SIR, or SRR)	48	1.01 (0.89, 1.15)	0.08
Colon	Relative Risk (SMR, SIR, or SRR)	19	1.10 (0.97, 1.25)	0.53
Rectum	Relative Risk (SMR, SIR, or SRR)	32	1.18 (1.01, 1.37)	0.94

[a]Warning displayed during estimation of the summary estimate indicates that results may not be stable due to the large range of sampling variance between included estimates.

Due to misclassification and heterogeneity of Cr(VI) exposure among and within the included studies, there may have been a decreased ability to detect an association if it existed. Although this analysis included studies that analyzed associations among occupational groups or subgroups with greater certainty of exposure to Cr(VI), variation in the prevalence, frequency and magnitude of exposure is likely within the exposure groups. Other factors that could contribute to the observed heterogeneity of risk estimates include presence of co-exposures and bias due to the use of occupational cohorts. Cancer risk in these industries is likely affected by prevalent exposures to other carcinogens in addition to Cr(VI), which would vary both within and across occupational groupings. As noted in Appendix Table C-51, two industry groupings with higher certainty of Cr(VI) prevalence, ferrochromium, chromate production, and stainless-steel workers, and chromium pigment exposed workers, had occupational settings characterized by different co-exposures, which argues against a strong common confounder. In some cases, authors did attempt to adjust for co-exposures or restrict the study population to minimize their effect. The majority of the studies estimated relative risk using SMRs, which also are subject to a bias toward the null due to the healthy worker effect. The summary effect estimates for esophageal and stomach cancers calculated using odds ratios from the few case-control studies was not subject to this bias and indicated a higher risk. However, these odds ratio estimates are based on very few studies and are highly uncertain.

Previous meta-analyses reported summary effect estimates for stomach cancer which ranged between 0.93 (Deng et al., 2019) to 1.27 (Welling et al., 2015). A statistically significant increase in risk of stomach cancer was reported from two of the previous five estimates (Welling et al., 2015; Cole and Rodu, 2005). This assessment's finding of no increased risk (summary relative risk of 1.01) is within the range of these previous estimates. Two of the five previous meta-analyses included estimates for cancers of the esophagus, colon and rectum (Deng et al., 2019; Gatto et al., 2010). This assessment's summary estimate of 1.08 for esophageal cancer was not significantly elevated, and was slightly less than that from Gatto et al. (2010). The effect estimate for colon cancer of 1.10 (95% CI: 0.97, 1.25), was close to the estimate reported by Deng et al. (2019). Finally, this assessment's estimate of rectal cancer risk was significantly elevated, and very similar to those previously reported (1.18, 95% CI: 1.01, 1.37), compared with 1.17 (Gatto et al., 2010) and 1.14 (Deng et al., 2019)).

Animal Evidence via the Oral Route of Exposure

Synthesis of neoplastic animal evidence

Neoplastic lesions following oral administration of Cr(VI) via drinking water were observed in the 2-year study conducted by NTP (2008) in both sexes of B6C3F1 mice and F344/N rats. This was the only animal study examining the potential for tumor development via the oral route of exposure and was rated as *high* confidence. An overview of the confidence classification for the GI histopathology reported in this study can be found in Section 3.2.2, Table 3-8 and in HAWC.

In this study, both sexes of F344/N rats exhibited an increased incidence of squamous cell carcinomas or papillomas in the oral cavity (mucosa or tongue), uncommon tumor types. Tumor incidence was statistically significant at the highest doses tested, 6.07 and 7.13 mg Cr(VI)/kg-d in male and female rats, respectively. The overall tumor incidence at the high dose was 14% in male rats and 22% in female rats (NTP, 2008), as compared to no tumors in control males and 2% incidence in females. There was also a nonsignificant, low incidence (4%) of oral cavity tumors in female rats receiving 2.6 mg Cr(VI)/kg-d. Microscopic examination of the tumors present in the oral cavity of rats indicated they were highly invasive, originating in the oral mucosa of the palate adjacent to the upper molar teeth with spread to the tongue, Harderian gland, the soft tissues surrounding the nose, and the brain (NTP, 2008).

In the same study, male and female B6C3F1 mice exhibited increased incidences of adenomas and carcinomas in the small intestine, with most tumors occurring in the duodenal section most proximal to the stomach. In male mice, there was a significant trend for increased incidence of adenoma and carcinomas in the small intestine. Statistically significant increases in adenomas or carcinomas were observed at doses ≥2.4 mg Cr(VI)/kg-d with an overall incidence of 40% at the high dose (NTP, 2008). Female mice also showed a significant trend for increased incidence of adenomas and carcinomas in the small intestine. At doses ≥3.24 mg Cr(VI)/kg-d, incidence of adenomas was statistically significantly increased and reached up to 44%. While most

tumors in both sexes were located in the duodenum (first section of the small intestine), female mice also showed a significant increase (10%) in overall incidence in the jejunum (middle section of the small intestine). Histopathological evaluation of the adenomas in mice were described as discrete, broad based and focally extensive; composed of irregular, elongated crypts; epithelial cells with oval to elongated nuclei; and increased mitotic activity (NTP, 2008). Carcinomas were characterized as extensive with invasion of the submucosa and/or muscularis mucosa; epithelial cells with round, oval, or elongated nuclei; and with atypical mitosis that was of greater extent than observed in adenomas.

The data for both species and sexes are summarized in Table 3-15 and Figure 3-15.

Table 3-15. Data on neoplastic lesions in a *high* confidence study of rats and mice (NTP, 2008)

Tumor type and species/sex		Administered mg/L, mg/kg-d Cr(VI)[a] and incidence/total				
Male B6C3F1 mice		**0 mg/L**	**5**	**10**	**30**	**90**
		0 mg/kg-d	**0.450**	**0.914**	**2.40**	**5.70**
Adenomas (duodenum)		1/50	0/50	1/50	5/50	15/50*
Carcinomas (duodenum)		0/50	0/50	0/50	2/50	3/50
Adenomas or carcinomas (duodenum, jejunum, or ileum)	Incidence / Total	1/50	3/50	2/50	7/50*	20/50*
	Incidence / Total (adj)[b]	1/50	3/49	2/49	7/50*	20/50*
Animals dead prior to day 365		0	1	1	0	0
Female B6C3F1 mice		**0 mg/L**	**5**	**20**	**60**	**180**
		0 mg/kg-d	**0.302**	**1.18**	**3.24**	**8.89**
Adenomas (duodenum)		0/50	0/50	2/50	13/50*	12/50*
Carcinomas (duodenum)		0/50	0/50	0/50	1/50	6/50*
Adenomas or carcinomas (duodenum, jejunum, or ileum)	Incidence / Total	1/50	1/50	4/50	17/50*	22/50*
	Incidence / Total (adj)[b]	1/49	1/50	4/49	17/50*	22/49*
Animals dead prior to day 365		1	0	1	0	1
Male F344 rats		**0 mg/L**	**5**	**20**	**60**	**180**
		0 mg/kg-d	**0.200**	**0.760**	**2.10**	**6.07**
Squamous cell carcinoma (oral mucosa)		0/50	0/50	0/49	0/50	6/49*
Squamous cell papilloma (oral mucosa)		0/50	0/50	0/49	0/50	1/49
Squamous cell carcinoma (tongue)		0/49	1/50	0/47	0/49	0/48
Squamous cell papilloma (tongue)		0/49	0/50	0/47	0/49	1/48
Squamous cell carcinoma or papilloma (oral mucosa or tongue)	Incidence / Total	0/50	1/50	0/49	0/50	7/49*
	Incidence / Total (adj)[b]	0/50	1/47	0/47	0/50	7/49*
Animals dead prior to day 365		0	3	2	0	0
Female F344 rats[c]		**0 mg/L**	**5**	**20**	**60**	**180**
		0 mg/kg-d	**0.248**	**0.961**	**2.60**	**7.13**
Squamous cell carcinoma (oral mucosa)		0/50	0/50	0/50	2/50	11/50*
Squamous cell carcinoma (tongue)		0/45	0/49	0/48	1/48	0/48
Squamous cell carcinoma (oral mucosa or tongue)	Incidence / Total	1/50	1/50	0/50	2/50	11/50*
	Incidence / Total (adj)[b]	1/50	1/50	0/50	2/50	11/50*
Animals dead prior to day 365		0	0	0	0	0

[a]Time-weighted average daily doses calculated from NTP water consumption data.
[b]Tumor incidences adjusted based on the number of animals surviving beyond 365 days. First tumor onset: 451 days for intestinal tumors in mice, and 506 days for oral tumors in rats (both occuring at the highest doses).
[c]For tissues where an effect was not observed, incidence data were not provided by NTP and were therefore not included in this table (i.e., there were no squamous cell papillomas in the oral cavity of female rats).
*Denotes significant difference from the control group reported by NTP (2008) using the Poly-3 test ($p < 0.05$).

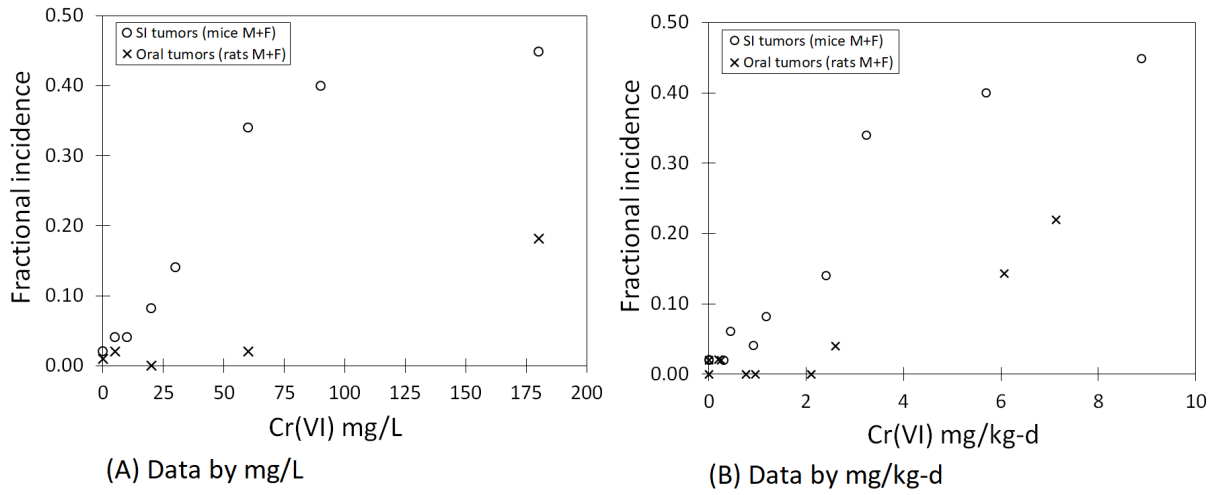

Figure 3-15. Fractional incidence of mice with adenomas or carcinomas in the small intestine (SI tumors), and fractional incidence of rats with squamous cell carcinomas or papillomas in the oral mucosa or tongue (oral tumors). Data presented on a basis of (A) administered mg/L Cr(VI), where incidence data for male and females were combined, and (B) administered mg/kg-d Cr(VI), where incidence data for males and females are separated due to differences in water intake and dose. For mice, both males and females were exposed to 5 mg/L, while all other nonzero doses differed between males and females. For rats, both males and females were exposed to the same mg/L Cr(VI) concentration levels. Incidence data adjusted for rodents surviving at least one year.

Notably, at the lower doses, incidences of specific neoplasms in the GI tract observed during the 2-year study exceeded NTP historical controls in both B6C3F1 mice and F344 rats. Therefore, some tumors which were not statistically significant versus concurrent controls at low doses may be biologically significant due to the increasing trend and low historical control incidence (Appendix D.5). Tumors of the oral cavity are rare (Ibrahim et al., 2021; Leininger and Schutten, 2018; Chandra et al., 2010). In the 2-year NTP (2008) bioassay, one squamous cell carcinoma was identified in the tongue of a male rat in the lowest dose group (0.2 mg Cr(VI)/kg-d), and in the tongue of a female rat at 2.6 mg Cr(VI)/kg-d. The historical controls for squamous cell carcinoma of the tongue are 0/1398 for male rats and 1/1350 for female rats (see Appendix D.5). The historical rates of squamous cell carcinomas and papillomas in the whole oral cavity in rats are less than 1% in both males and females. In the 2-year bioassay, there was an increasing trend in these tumor types in both male and female rats (Figure 3-15), with a 22% incidence in female rats at the highest dose. Tumors of the small intestine of mice are also rare (historical rates of 2.3% and 0.67% in males and females, respectively). These tumors were observed in all exposed groups of mice (including 3/49 at the lowest dose in males), with an incidence of ≥40% in the highest dose groups in both sexes. One tumor each was observed in the control groups of male and female mice (leading to a 2% incidence for controls). In general, historically, rats are more prone to oral cancer development than mice, and mice are more prone to neoplasia in the small intestine (Ibrahim et al.,

2021; Chandra et al., 2010) (Appendix D.5). The reason is unknown, but likely multifactorial in nature, possibly involving differences in the microbiome (Ibrahim et al., 2021).

3.2.3.3. *Mechanistic Evidence (all routes)*

Cr(VI) is a human lung carcinogen when inhaled. When ingested, Cr(VI) has been shown to cause tumors in the GI tract in animals exposed in drinking water (NTP, 2008) and there are indications from a meta-analysis of chromate-exposed workers that Cr(VI) are carcinogenic in the GI tract in humans (see above). Evidence relevant to the potential key events and pathways involved in Cr(VI)-induced cancer via oral or inhalation exposures was systematically identified (Section 1.2) and is presented in detail in Appendix C.3.2 organized by the key characteristics of carcinogens (Smith et al., 2016). The key characteristics of Cr(VI) with the largest evidence bases and most relevant study designs are DNA reactivity (electrophilicity/formation of DNA adducts), genotoxicity, altered DNA repair processes and genomic instability, epigenetic effects, oxidative stress, and altered cell division and death. This evidence, along with the evidence of tumors and preneoplastic lesions from animal bioassays and from gene expression (Appendix C.3.3) and toxicogenomic studies (Appendix C.3.4), informed the identification of the steps and key events involved in Cr(VI)-induced cancer as described in EPA's cancer guidelines (U.S. EPA, 2005a).

There are multiple mechanistic processes induced by Cr(VI) exposure that appear to contribute to carcinogenesis. The large majority of the mechanistic evidence relevant to interpretations of upstream mechanistic processes induced by Cr(VI) that may lead to tumorigenesis has been summarized in Appendix C.3 and in Section 3.2.3.4. The key events identified to be involved in the carcinogenic process induced by Cr(VI) are the distribution, cellular uptake, and intracellular reduction of Cr(VI); the DNA reactivity of chromium and the formation of Cr-DNA adducts; oxidative stress and free radical-induced cytotoxicity and DNA damage; epigenetic modifications; altered DNA repair; the silencing of tumor suppressor genes and the activation of oncogenes; genomic instability; gene and chromosomal mutation; the suppression of apoptosis; cytotoxicity and degenerative cellular changes; cell proliferation and regenerative hyperplasia; and chronic inflammation. The evidence for these key events is summarized in the next section, 3.2.3.4.

The studies informing these key events were not evaluated for risk of bias, reporting, and sensitivity concerns using predefined metrics. However, a set of studies with designs best suited to examining whether and to what extent Cr(VI)-induced tumorigenesis involves a mutagenic MOA were prioritized and subject to an additional level of review. This includes studies measuring gene or chromosomal mutation endpoints in occupationally exposed humans and studies in experimental animals in inhalation or oral exposure scenarios (see below). This is because the results of the analyses of whether Cr(VI) acts via a mutagenic MOA for cancer can influence dose-response decisions, including the application of age-dependent adjustment factors (ADAFs) and low-dose linear extrapolation (U.S. EPA, 2005b). It is also for this reason that this MOA analysis includes consideration of both GI and lung tumors; although the hazard for lung cancer is not being revisited (see Section 3.2.3.1), a determination of whether a mutagenic MOA is applicable to lung

tumors is important to consider for dose-response. The summary and evaluation of the mechanistic evidence most informative to evaluating the role of mutagenicity is synthesized in the following sections. The inferences drawn from these syntheses are then used to construct and analyze a mutagenic MOA for carcinogenesis; the MOA analyses for a mutagenic MOA and whether a mutagenic MOA could be secondary to tissue injury and compensatory proliferation induced by Cr(VI) are presented in Section 3.2.3.4, "Cancer mode-of-action summary."

Evidence informing a mutagenic MOA

A mutation is a permanent, transmissible change in the genetic material of an organism. Mutations can be caused by alterations in the DNA sequence of a gene, as well as structural (clastogenic) and numerical (aneugenic) chromosome alterations (Eastmond et al., 2009). Genotoxicity is a more comprehensive term, referring to the ability of an exogenous agent to alter genetic material. Some genotoxicity assays directly measure mutations, while others measure DNA damage; proficient DNA repair of these genetic alterations depends on many factors including the type of genetic damage and the repair capacity of the individual. Although both terms will be used in the following sections, the more inclusive term "genotoxicity" will be used when discussing evidence for a mutagenic MOA in a broader context. Consideration of both types of genotoxicity evidence and a broad survey of multiple genotoxicity endpoints, when available, is important for a comprehensive characterization of an agent's genotoxicity and the underlying genotoxic processes.

A large body of evidence is available to inform the genotoxicity of Cr(VI). Many genotoxicity studies of Cr(VI) were conducted in test systems primarily used to screen substances for genotoxic potential, which are useful but also include endpoints measuring genetic damage that may not represent damage that is transmissible to daughter cells, or that use exposure methods that are expected to result in higher concentrations of Cr(VI) at the cell membrane, including i.p. administration and in vitro studies, leading to a greater quantity of Cr(VI) being taken up by the cell and reduced to Cr(III). These studies have largely shown that intracellular Cr(III) can form DNA adducts (reviewed in Zhitkovich (2011)) and is mutagenic (reviewed in Chen et al. (2019), Wise et al. (2018) and Nickens et al. (2010)). This section is focused on the phenotypic evidence for Cr(VI)-induced genotoxicity; the evidence for the mechanisms underpinning this genotoxicity, including cellular uptake and reduction of Cr(VI) and the formation of Cr-DNA adducts and oxidative DNA lesions, is summarized in the key events for the cancer MOA in Section 3.2.3.4. All studies informing genotoxic mechanisms are considered, but a more specific and critical analysis below focuses on evidence that most directly informs the ability of Cr(VI) to cause mutations in exposed humans. Namely, using the study prioritization and evaluation criteria described in Appendix C.3.2.2, this analysis focuses on studies that use assays to detect transmissible genetic damage (i.e., gene mutation, micronuclei, and chromosomal aberrations) observed in exposed humans or in mammalian test systems in vivo utilizing routes of exposure more applicable to humans (i.e., oral and inhalation).

Human study evaluation summary

Studies of occupationally or environmentally exposed humans were considered to be most relevant to a mutagenic MOA analysis for cancer if they included measures of gene mutation (prior to tumorigenesis), micronuclei induction, or chromosomal aberrations. Human studies were only considered if they included a comparison or referent population exposed to Cr(VI) at lower levels (or no exposure/exposure below detection limits) or for shorter periods of time. Twenty-nine studies of chromosomal aberrations and/or micronuclei in humans were identified according to these prioritization considerations (see Appendix C.3.2.2) and evaluated for reporting quality, risk of bias and sensitivity. Six studies were considered but deemed *uninformative* due to critical deficiencies in either the exposure or outcome domain (Wultsch et al., 2017; Coelho et al., 2013; Sellappa et al., 2010; Hilali et al., 2008; Cid et al., 1991; Sarto et al., 1990) and are not discussed further. The confidence judgments of the 23 informative studies, all conducted in workers occupationally exposed to Cr(VI) that are expected to primarily be inhalation exposures, are summarized in Table 3-16. All of the included studies were cross-sectional in design, comparing individuals employed in occupations with known potential for chromium exposure to referent groups, with the specific occupations, geographic locations, and exposure measurement methods are summarized in Table 3-16. No oral exposure studies in humans were identified.

All studies were categorized as *low* or *medium* confidence. Among *low* confidence studies, common reasons for decreased confidence ratings included small sample size/low power (Linqing et al., 2016; Wultsch et al., 2014; Medeiros et al., 2003; Benova et al., 2002; Vaglenov et al., 1999; Deng et al., 1988; Husgafvel-Pursiainen et al., 1982; Sarto et al., 1982), presence of co-exposures to other occupational hazards that may also contribute to the observed genotoxicity (e.g., nickel) not accounted for in the design or analysis (Wultsch et al., 2014; Qayyum et al., 2012; Iarmarcovai et al., 2005), residual confounding due to minimal or no control for covariates (Balachandar et al., 2010; Vaglenov et al., 1999; Koshi et al., 1984), limitations in outcome assessment techniques or inadequate reporting (Qayyum et al., 2012; Balachandar et al., 2010; Danadevi et al., 2004; Koshi et al., 1984; Littorin et al., 1983; Sarto et al., 1982), and insufficient description to allow for evaluation of potential for bias (including selection bias) (Linqing et al., 2016; Qayyum et al., 2012; Balachandar et al., 2010; Halasova et al., 2008; Iarmarcovai et al., 2005; Danadevi et al., 2004; Maeng et al., 2004; Medeiros et al., 2003; Benova et al., 2002; Koshi et al., 1984; Sarto et al., 1982). Among *medium* confidence studies, the most common reason for decreased confidence rating was insufficient description to allow for evaluation of potential for bias (including selection bias) (Long et al., 2019; El Safty et al., 2018; Hu et al., 2018; Halasova et al., 2012).

For all studies, exposure to chromium was inferred based on occupational group. Given the likelihood of chromium exposure in the industries evaluated, an exposure assessment that did not include a precise estimate of exposure levels was not identified as a primary limitation in most of these studies for consideration with respect to mechanistic interpretations. However, lack of certainty about differentiation of exposure between comparison groups (including the potential for

exposure among "controls") was a concern in several studies (Halasova et al., 2012; Vaglenov et al., 1999; Migliore et al., 1991; Deng et al., 1988). In all but two studies (Sudha et al., 2011; Migliore et al., 1991), chromium biomarker and/or air concentrations were also measured; these data served to confirm that exposure occurred and provided context for results, but these measurements were not a requirement in the evaluation criteria.

Table 3-16. Summary of included human cross-sectional occupational studies for Cr(VI) mutagenic effects and overall confidence classification [high (H), medium (M), low (L)] by outcome. Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Exposure Measurement/Cr Validation Measures	Gene mutation	Chromosomal aberrations	Micronuclei
El Safty et al. (2018)	Chrome electroplating	Egypt	Job category/ serum samples	-	-	M
Halasova et al. (2012)	Welding	Slovak Republic	Job category/blood samples	-	M	-
Hu et al. (2018)[a]	Unspecified factory work with exposure to chromate	China	Job category/ blood and air samples	-	-	M
Long et al. (2019)	Chromate production	China	Job category/blood samples	-	-	M
Sudha et al. (2011)	Welding	India	Job category	-	-	M
Balachandar et al. (2010)	Tannery	India	Job category/urine and air samples	-	L	L
Benova et al. (2002)	Chrome electroplating	Bulgaria	Job category/urine and air samples	-	L	L
Danadevi et al. (2004)	Welding	India	Job category/ blood samples	-	-	L

Author (year)	Industry	Location	Exposure Measurement/Cr Validation Measures	Gene mutation	Chromosomal aberrations	Micronuclei
Deng et al. (1988)	Chrome electroplating	China	Job category/ air, hair, and stool samples	-	L	-
Halasova et al. (2008)	Welding	Slovak Republic	Job category/ blood samples	-	L	-
Husgafvel-Pursiainen et al. (1982)	Welding	Finland	Job category/ urine samples	-	L	-
Iarmarcovai et al. (2005)	Welding	France	Job category/blood and urine samples	-	-	L
Koshi et al. (1984)	Stainless-steel welding	Japan	Job category/ urine samples	-	L	-
Linqing et al. (2016)	Chrome electroplating	China	Job category/blood samples	-	-	L
Littorin et al. (1983)	Stainless-steel welding	Sweden	Job category/ urine and air samples	-	L	U
Maeng et al. (2004)	Chrome electroplating and buffing	South Korea	Job category/urine, blood, and air samples	-	L	-
Medeiros et al. (2003)	Stainless-steel welders; Tannery	Portugal	Job category/plasma and urine samples	-	-	L
Migliore et al. (1991)	Tannery	Italy	Job category	-	-	L
Qayyum et al. (2012)	Chrome electroplating	India	Job category/plasma samples	-	-	L
Sarto et al. (1982)	Chrome electroplating	Italy	Job category/urine samples	-	L	-

Author (year)	Industry	Location	Exposure Measurement/Cr Validation Measures	Gene mutation	Chromosomal aberrations	Micronuclei
Vaglenov et al. (1999)	Hydraulic machinery; Chrome electroplating	Bulgaria	Job category/air, red blood cells, urine samples	-	-	L
Wultsch et al. (2014)	Chrome electroplating	Austria	Job category/whole blood samples	-	-	L
Xiaohua et al. (2012)	Chromate production	China	Job category/urine, blood, air samples			L

[a]Two other studies by the same group (Li et al., 2014a; Li et al., 2014b) reported the same micronucleus frequency data and were tagged as "duplicate data" supplemental material.

Synthesis of human genotoxicity evidence

Among the 23 informative studies prioritized for evaluating mutagenicity, 16 evaluated micronucleus incidence and 10 evaluated chromosomal aberrations (three studies evaluated more than one of these endpoints). The study details are summarized in Table 3-17 (more detailed summaries can be found in Appendix Table C-54).

Table 3-17. Associations between Cr(VI) exposure and prioritized genotoxicity outcomes in epidemiology studies[a]

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
El Safty et al. (2018) *Medium* confidence	Cross-sectional study in Egypt Exposed: 41 electroplating workers Referents: 41 administrative workers	26.68 (11.21)	Air (mg/m^3) *Total Cr* Exposed: median: 15.5 (IQR: 19.0) Referents: median: 0.2 (IQR: 0.4) Blood (µg/L) Exposed: 8.5 (1.3) Referents: 4.1 (1.4)	In exfoliated buccal cells: ↑ MN in exposed compared to controls ($p < 0.001$) ↑ serum Cr correlates with ↑ MN ↑ serum 8-OHdG in exposed compared to controls ($p < 0.001$)
Halasova et al. (2012) *Medium* confidence	Cross-sectional study in Slovak Republic Exposed: 73 welders Referents: 73 individuals without known exposures	10.2 (1.7)	Blood (µmol/L) *Total Cr* Exposed: 0.07 (0.04) Referents: 0.03 (0.007)	In cultured lymphocytes: No significant differences in CAs between exposed and control groups ↑ CAs in individuals with Gln/Gln genotype compared to Arg/Gln or Arg/Arg genotypes in XRCC1 Arg399Gln; more pronounced in Cr-exposed workers ($p = 0.01$) (no correlation with XRCC3 polymorphisms)
Hu et al. (2018) *Medium* confidence	Cross-sectional study in China Exposed: 87 workers at factory with chromate exposure Referents: 30 administrative workers	Median: 5.0 IQR: 7.0	Air ($\mu g/m^3$) Exposed: median: 15.5 (IQR:19.0) Referents: median: 0.2 (IQR: 0.4) Blood (µg/L) Exposed: GM: 8.5 (1.3) Referents: GM:4.1(1.4)	↑ MN in peripheral lymphocytes in exposed workers compared with referent
Long et al. (2019) *Medium* confidence	Cross-sectional study in China Exposed: 120 chromate production facility workers Referents: 97 unexposed workers at the same factory	14.57 (5.85)	Blood (µg/L) Exposed: median: 2.81 (IQR: 3.86) Referents: median: 0.99 (IQR: 1.21)	↑ MN frequency ratio in lymphocytes of exposed Interactions between Cr exposure and MN frequency in lymphocytes for some SNPs

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
Sudha et al. (2011) *Medium* confidence	Cross-sectional study in India Exposed: 66 welders Referents: 60 general population controls	Range: 5–20	NR	In exfoliated buccal cells: ↑ MN frequency and comet tail length in welders compared to controls; increased with duration of work ($p < 0.05$)
Balachandar et al. (2010) *Low* confidence	Cross-sectional study in India Exposed 1: 36 directly exposed (DE) through tannery work Exposed 2: 36 indirectly exposed (IE) through residential proximity to tanneries Referents: 36 unexposed individuals	DE (tannery) workers (% by duration) 0–5: 17% 5–10: 33% 10–15: 36% 15–20: 11% 20–25: 3%	Air (mg/m^3) *Cr(VI)* DE 0.021 (0.003) IE: 0.013 (0.005) Referents: 0.006 (0.001) Urine DE: 2.11 (1.01) IE: 1.81 (0.88) Referents: 0.54 (0.39) (*Units not provided*)	In cultured lymphocytes: ↑ CAs in DE group compared to IE group and controls ↑ MN among directly exposed subjects compared to indirectly exposed & controls; further elevated in those with longer duration of exposure ↑ mean tail length for comet assay in DE group compared to IE group and controls
Benova et al. (2002) *Low* confidence	Cross-sectional study in Bulgaria Exposed: 15 chrome-plating workers Referents: 23 individuals (15 workers and 8 rural residents)	N by duration: 2–5: 3 6–10: 1 11–15: 4 16–20: 4 >20: 3	Air (mg/m^3) *Cr(VI)* High exposed workers: 0.0249 (SE: 0.004) Low exposed workers: 0.0075 (SE: 0.001) Referents: 0.0004 (SE: 0) Urine (µg/L) High exposed workers: 104.22 (SE: 27.51) Low exposed workers: 18.63 (SE: 3.16) Referents: 1.18 (SE: 0.23)	In cultured lymphocytes and exfoliated buccal cells: No significant difference in frequencies of CAs or SCEs in exposed workers compared to controls ↑ MN in workers compared to controls (lymphocytes: $p < 0.01$; buccal: $p < 0.001$)
Danadevi et al. (2004) *Low* confidence	Cross-sectional study in India Exposed: 102 welders Referents: 102 general population controls	Range: 1–24	Blood (µg/L) Exposed: 151.65 (SD not provided) Referents: 17.86 (SD not provided)	↑ MN in exfoliated buccal cells compared to controls ($p < 0.001$), correlated with duration of work, age, and Cr level in blood ↑ mean comet tail length in whole blood cells compared to controls ($p < 0.001$)

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
Deng et al. (1988) *Low* confidence	Cross-sectional study in China Exposed 1: 7 electroplating workers exposed to chromium Exposed 2: 7 electroplating workers exposed to nickel Referents: 10 officer workers	12.8 (range: 4–18)	Air (mg/m^3) *Total Cr* Workers: 8×10^6 (SE: 3.7×10^6) Stool (µg/g) Workers: 8.5 (SE: 3.2) Hair (µg/g) Workers: 35.7 (11.5)	In cultured lymphocytes: ↑ CAs in chromium workers compared to nickel workers & controls ↑ SCE in chromium & nickel workers compared to controls
Halasova et al. (2008) *Low* confidence	Cross-sectional study in Slovak Republic Exposed: 39 welders Referents: 31 individuals without known exposures	10.2 (1.7)	Blood (µmol/L) *Total Cr* Exposed: 0.07 (0.04) Referents: 0.03 (0.007)	In cultured lymphocytes: Nonsignificant ↑ CAs in exposed compared to control groups ↑ CAs ($p < 0.05$) in lymphocytes in individuals with Gln/Gln genotype compared to Arg/Gln or Arg/Arg genotypes in XRCC1 Arg399Gln (no correlation with XRCC3 polymorphisms)
Husgafvel-Pursiainen et al. (1982) *Low* confidence	Cross-sectional study in Finland Exposed: 23 welders Referents: 22 employees at printing company	21 (10)	Urine (µmol/L) *Total Cr* Exposed: range: 0.20–1.55	In cultured lymphocytes: No significant differences in frequency of CAs or SCEs between welders and controls

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
Iarmarcovai et al. (2005) *Low* confidence	Cross-sectional study in France Exposed: 60 welders n = 27 working in areas "without any collective protection device" n = 33 working in places with "smoke extraction systems Referents: 30 office workers	Range: 0.5–45	Blood (µg/L) Exposed: 123.8–145.8 (58.8–87.7)[c] Referents: 92.0 (15.0) Urine (µg/g creatinine) Exposed: 18.6–33.0 (11.0–21.4)[c] Referents: 12.8 (6.6)	In cultured lymphocytes: ↑ MN in non-protected welders compared to controls (p = 0.03) ↑ mean comet tail length in welders at the end of the work week (p < 0.001); not significant at the start of the week ↑ mean comet tail length in individuals with Gln/Gln genotype compared to Arg/Gln or Arg/Arg genotypes in XRCC1 Arg399Gln (no correlation with XRCC3 polymorphisms)
Koshi et al. (1984) *Low* confidence	Cross-sectional study in Japan Exposed: 51 stainless-steel welders Referents: 33 office/research workers	12 (range: 5–20)	Urine (µg/L) Exposed: 9.8 (9.2) Referents: 4.2 (1.2) µg/L	In cultured lymphocytes: ↑ CAs and SCEs in welders compared to controls
Linqing et al. (2016) *Low* confidence	Cross-sectional study in China Exposed: 29 chrome-plating workers Referents: 29 workers without chromate exposure history	NR	Blood (µg/L) Exposed: 15.2 (range: 2.1–42) Referents: 4.6 (range: 0.2–28)	In cultured lymphocytes: ↑ MN frequencies in workers compared to controls (p = 0.0048) No correlation between blood Cr concentration and MN ↓ methylation of MT-TF and MT-RNR1 genes in mitochondrial DNA correlated with blood Cr

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
Littorin et al. (1983) *Low* confidence	Cross-sectional study in Sweden Exposed: 24 stainless-steel welders Referents: 24 matched individuals without occupational mutagenic exposures	19 (range: 7–41)	Air (mg/m^3) *Cr VI* Exposed: 0.055 (range: 0.005–0.321) Urine (µmol/L) Exposed: 47 (range: 5–155) Referents: 1.5 (range: <0.4–7.0)	In cultured lymphocytes: No significant differences in CAs or SCEs between exposed and control groups No significant differences in MN between exposed and control groups
Maeng et al. (2004) *Low* confidence	Cross-sectional study in South Korea Exposed: 51 male chrome-plating/buffing workers Referents: 31 office workers	9.1 (range: 0–40)	Air (mg/m^3) *Cr VI* Exposed: GM: 0.0032 (range: 0.0003–0.09) Referents: GM: 3×10^{-5} (range: 1.4×10^{-5}–6.1×10^{-5}) Blood (µg/dL) Exposed: GM: 0.86 (range: 0.11–8.99) Referents: GM: 0.17 (range: 0.00–0.67) Urine (µg/g creatinine) Exposed: GM: 12.82 (range: 0.66–8.74) Referents: GM: 3.39 (range: 0.40–9.04)	In cultured lymphocytes: Nonsignificant ↑ CAs detected by solid Giemsa staining in exposed compared with unexposed that were statistically correlated with higher blood Cr ↑ CAs with ↑ frequency of chromosome translocations in exposed compared with unexposed ($p < 0.01$) detected by FISH ↑ MDA in blood plasma in exposed compared to controls ($p < 0.01$)
Medeiros et al. (2003) *Low* confidence	Cross-sectional study in Portugal Exposed 1: 5 welders Exposed 2: 33 tannery workers Referents: 20–30 unexposed individuals	NR	Plasma (µg/L) Tannery workers: 2.43 (2.11) Welders: 1.55 (0.67) Referents: 0.41 (0.11) Urine: (µg/g creatinine) Tannery workers: 2.63 (1.62) Welders: 1.90 (0.37) Referents: 0.70 (0.38)	In cultured lymphocytes: ↑ MN in tannery workers compared to controls ($p < 0.01$) Nonsignificant ↑ MN in welders (n = 5) ↑ DNA-protein crosslinks in tanners ($p < 0.001$) and welders ($p < 0.05$) compared to controls
Migliore et al. (1991) *Low* confidence	Cross-sectional study in Italy Exposed: 17 tannery workers and 2 reference groups from different industries	NR	NR	No effects on MN frequency in cultured lymphocytes

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
Qayyum et al. (2012) *Low* confidence	Cross-sectional study in India Exposed: 100 electroplating workers (grouped by length of work) Referents: 50 individuals with no known exposure to nickel or chromium	Group 1: range: 1–9 Group 2: range: 10–25	Plasma (µg/L) Group 1: 2.9 (0.8) Group 2: 1.7 (0.6) Referents: 0.6 (0.8)	In buccal cells of Group II compared to Group I, and in Group III compared to Group II: ↑ MN frequency ($p < 0.05$) MN also correlated with Cr levels in plasma ($p < 0.01$)
Sarto et al. (1982) *Low* confidence	Cross-sectional study in Italy Exposed: 38 plating factory workers (bright plating and hard plating) Referent 1: 35 sanitary workers Referent 2: 14 healthy blood donors	Hard plating: 7 (3) Bright plating: 9 (11)	Urine (µg/g creatinine) Exposed—Hard plating: 10.0 (7.5) Exposed—Bright plating: 6.1 (2.8) Referents: 1.9 (1.4)	In cultured lymphocytes: ↑ CAs (mostly CSAs) among all exposed bright platers ($p < 0.001$) and hard platers ($p < 0.01$) compared to controls ↑ SCEs in hard platers compared to blood donors
Vaglenov et al. (1999) *Low* confidence	Cross-sectional study in Bulgaria Exposed: 30 hydraulic machinery workers (grouped by high and low exposure) & 10 hospitalized electroplating workers Referents: 18 administrative workers	Overall range: 4–25 High exposed mean: 11.63 Low exposed mean: 10.44	Air (mg/m^3) High exposed: 0.083 (SE: 0.010) Low exposed: 0.043 (SE: 0.01) Referents: 0.0003 (SE: 0.0001) Erythrocytes (µg/L) High exposed: 8.40 (SE: 1.93) Low exposed: 4.31 (SE: 1.03) Referents: 0.57 (SE: 0.05) Urine (µg/L) High exposed: 5.0 (SE: 1.52) Low exposed: 3.97 (SE: 1.98) Referents: 0.49 (SE: 0.06)	↑ MN and binucleated cells carrying MN in lymphocytes of exposed compared to control Correlations of Cr measured in air, erythrocytes and urine, with higher MN in lymphocytes

Reference	Population	Duration of work in exposed group (mean (SD) yrs)	Cr measurements (mean (SD) unless otherwise indicated)	Endpoints[b]
Wultsch et al. (2014) *Low* confidence	Cross-sectional study in Austria Exposed: 22 chrome-plating workers Referents: 22 jail warden controls	NR	Blood (µg/L) Exposed: 2.3 (1.5) Referents: 0.2 (0.2)	In exfoliated cells of exposed chrome platers compared to referent: ↑ MN frequency in nasal cells (p = 0.005) No significant effect on MN frequency in buccal cells (23% increase; p = 0.516) ↑ nuclear anomalies in buccal and nasal cells
Xiaohua et al. (2012) *Low confidence*	Cross-sectional study in China Exposed: 79 chromate production workers Referents: 112 peasant volunteers without occupational chromate exposure	Mean: 14.89 SE: 8.65	Air (µg/m^3) Exposed: 13.01 (range:1.03–56.60) Referents: 0.073 (range: 0.023–0.235) Blood (µg/L) Exposed: 9.19 (range: 1.17–51.88) Referents: 3.44 (range: 0.25–22.51) Urine (µg/g creatinine) Exposed: 17.03 (range: 2.78–97.23) Referents: 2.49 (range: 0.39–26.82)	↑ MN in binucleated blood cells in exposed group compared to controls Moderate correlations (0.353–0.517) between BNMN and Cr concentrations in blood, urine, air

GM = geometric mean; IQR = interquartile range; SE = standard error; CA = chromosomal aberration; MN = micronuclei; NR = not reported.

[a]Studies presented by study confidence (high to low) first, then alphabetically by author.

[b]Some endpoints reported by the same study but not included in the PECO are also included here for context. p-values are added to provide additional context but should not be the sole focus for interpretation.

[c]This study reported subgroup means and SD; therefore, this table reports the range of means and the range of SDs for these groups.

1

Micronuclei

Micronuclei are formed when dividing cells contain whole chromosomes or acentric chromosome fragments that have lagged behind during anaphase, indicating aneuploidy or the presence of chromosomal aberrations. Additional procedures to detect the presence of a centromere in the micronucleus can distinguish between loss of a whole chromosome or chromosome fragments. All prioritized studies in humans focused on the detection of micronuclei or chromosomal aberrations in peripheral blood lymphocytes or exfoliated nasal or buccal cells (epithelial cells inside the mouth/cheek). In humans, it has been shown that an increased frequency of micronuclei in circulating blood is positively associated with an increased risk of cancer (Bonassi et al. (2011b; 2007)). In addition, micronuclei detected in exfoliated epithelial cells from the oral buccal or nasal mucosa is an effective measure of genetic damage in directly exposed tissues (Bonassi et al., 2011a).

Among the 16 studies evaluating micronuclei, four were rated as *medium* confidence and 12 were rated as *low* confidence. All four of the *medium* confidence studies reported increased micronuclei, with two studies reporting these increases in lymphocytes (Long et al., 2019; Hu et al., 2018), and two reporting increases in buccal cells (El Safty et al., 2018; Sudha et al., 2011). These studies included populations from several industries with chromium exposure including electroplating, chromate production, and welding. While these studies compared groups defined by job category, three of the four studies augmented the exposure assessment by including data from supplemental biomarker and/or air measures that showed total Cr levels were higher in exposed workers and in exposure settings, confirming that exposures occurred and providing context for the positive results (Long et al., 2019; El Safty et al., 2018; Hu et al., 2018) (see Table 3-17).

Among the 11 *low* confidence studies, there were ten that reported increased micronuclei for at least one cell type. Three evaluated buccal cells (Qayyum et al., 2012; Danadevi et al., 2004; Benova et al., 2002), six evaluated lymphocytes and/or leukocytes in peripheral blood (Linqing et al., 2016; Balachandar et al., 2010; Iarmarcovai et al., 2005; Medeiros et al., 2003; Benova et al., 2002; Vaglenov et al., 1999), and one evaluated nasal cells (Wultsch et al., 2014) (this study also reported a slight nonsignificant increase in micronuclei in buccal cells). These studies were comprised of populations exposed to chromium via welding, electroplating, hydraulic machinery, and tanneries. These studies also confirmed exposure in biomarker and/or air measures of total Cr or Cr(VI), though Linqing et al. (2016) did not detect a significant correlation between the increased blood Cr levels and statistically significantly increased micronucleus frequency in exposed workers (Table 3-17). As described in Appendix C.3.3., the potential direction of bias in these *low* confidence studies could not be determined.

One *low* confidence study reported no significant effects on micronucleus endpoints. In this study, Migliore et al. (1991), there is uncertainty regarding the potential for chromium exposure among the tannery workers evaluated and no accompanying biomarker measurements to provide confirmation; misclassification of individuals with regards to exposure group may produce bias

towards the null. In the absence of quantitative measures of exposure, it cannot be determined whether a negative result reflects low exposures, a lack of mutagenicity, or both.

Dose-response concordance for the observed increases in micronuclei was observed in several studies, most reliably based on correlations between Cr levels measured in blood and micronuclei in buccal cells in the *medium* confidence study by El Safty et al. (2018) and also in the *low* confidence studies by Qayyum et al. (2012) and Danadevi et al. (2004). Danadevi et al. (2004) also noted a correlation between Cr levels and duration of work and age. A correlation between work duration and increased micronucleus frequency was also noted in buccal cells in the medium confidence study by Sudha et al. (2011) and in lymphocytes in the low confidence study by (Balachandar et al., 2010).

Several of these studies also reported other significantly increased systemic genotoxicity markers in exposed workers that may be coherent with the observed micronuclei increases, including serum 8-OHdG (El Safty et al., 2018) and comet tail length in blood cells (Sudha et al., 2011; Danadevi et al., 2004).

Overall, all four *medium* confidence studies across different study populations and industrial settings (Table 3-16) and covering both lymphocytes and exfoliated epithelial cells provide evidence for an association between chromium exposure and increased micronuclei. These results are supported by the large majority of the available *low* confidence studies. Despite their limitations, *low* confidence studies provide supporting evidence for this endpoint in conjunction with the conclusions from *medium* confidence studies. In addition, when looking broadly across studies and evaluating the evidence base as a whole, concerns about any particular study deficiency is attenuated given that ten of the 11 *low* confidence studies demonstrated increases in micronuclei despite differences in population and exposure scenarios.

Chromosomal aberrations

Structural or numerical chromosomal aberrations, observable during metaphase in cells undergoing mitosis, are typically detected using simple, solid-staining techniques that allow visual identification of chromosome and chromatid breaks, but do not detect translocations or other more complex forms of chromosomal damage. Use of G-banding techniques or molecular fluorescent probes (e.g., FISH) increase the type and complexity of detectable cytogenetic damage. In humans, it has been shown that an increased frequency of chromosomal aberrations in circulating blood is positively associated with an increased risk of cancer (Bonassi et al., 2008; Norppa et al., 2006).

All included studies evaluating chromosomal aberrations were rated as *low* confidence except for one *medium* confidence study, Halasova et al. (2012), that identified chromosomal aberrations only within genetically susceptible populations but did not identify differences between the broader exposed and control groups. It should be noted, however, that a concern for bias towards the null due to potential insensitivity was identified for this study (see Appendix C.3.2.2). The mean levels of blood chromium among the exposed group in this study were low (0.07 µmol/L = 3.64 µg/L) and within the range reported for the referent groups in other studies of

chromosomal aberrations (e.g., Maeng et al. (2004): 2.0 μg/L) and micronuclei (e.g., Linqing et al. (2016): 4.6 μg/L). Lack of control for potential confounders is also a concern in this study (Halasova et al., 2012).

Among the nine *low* confidence studies, six reported increased chromosomal aberrations among exposed compared to unexposed individuals (Balachandar et al., 2010; Halasova et al., 2008; Maeng et al., 2004; Deng et al., 1988; Koshi et al., 1984; Sarto et al., 1982). These studies examined individuals exposed to chromium in a range of settings, such as tanneries, mining, electroplating, and welding. While several studies had deficiencies that pose substantial concern for bias, such as limited evaluation of confounders or potential for selection bias (Koshi et al., 1984; Sarto et al., 1982), others had deficiencies that primarily relate to sensitivity, such as small sample size and unclear differentiation between exposure groups (Balachandar et al., 2010; Halasova et al., 2008; Deng et al., 1988). Identification of effects on chromosomal aberrations despite sensitivity concerns in these studies that may bias results towards the null can provide stronger evidence of effect despite the individual overall study quality ratings of *low*.

Three *low* confidence studies evaluating populations of welders or chrome-plating workers reported no changes in chromosomal aberrations in exposed individuals compared to controls (Halasova et al., 2008; Benova et al., 2002; Littorin et al., 1983). It should be noted that two of these studies may have limited power to detect the outcome of interest due to small sample size (Benova et al., 2002; Husgafvel-Pursiainen et al., 1982).

Overall, while the evidence base is mostly consistent regarding the association between chromium exposure and chromosomal aberrations across a variety of exposure scenarios, biomarkers, and geographic regions, these observations are only available from studies rated as *low* confidence and a single medium confidence study with mixed results. Although considering the entire evidence base mitigates concerns about any particular deficiency in a single *low* confidence study and some of these studies detected effects despite limitations in power and sensitivity (Coelho et al., 2013; Balachandar et al., 2010; Halasova et al., 2008; Deng et al., 1988), it is difficult to draw definitive judgments from the predominantly low confidence evidence base on chromosomal aberrations.

Supporting genotoxicity evidence

In addition to the studies of gene and chromosome mutation described above, other types of genotoxicity studies conducted among humans exposed occupationally or environmentally to Cr(VI) are considered supporting evidence for the ability of Cr(VI) to cause genetic damage in exposed workers. These are biomonitoring DNA damage assays conducted on peripheral blood that measure DNA strand breaks, adducts, crosslinks, or other DNA damage and repair-related endpoints (e.g., sister chromatid exchange). These studies are summarized in Appendix Table C-49. They did not undergo formal study evaluation unless they included other endpoints that met the mutagenic prioritization criteria.

DNA damage in exposed humans

Seven of eight studies of exposed chromium industry workers detected significant increases in DNA strand breaks in peripheral blood using the comet assay; seven of these studies also confirmed exposures by detecting higher Cr levels in air and/or biomarkers compared to referents (Wang et al., 2012b; Sudha et al., 2011; Zhang et al., 2011; Balachandar et al., 2010; Iarmarcovai et al., 2005; Danadevi et al., 2004; Gambelunghe et al., 2003; Gao et al., 1994). These tests provide supporting evidence for increased genetic damage following Cr(VI) exposure, though they do not anticipate the proportion of DNA strand breaks that could lead to mutation. Five studies evaluated DNA-protein crosslinks, which are considered biomarkers for the genotoxic effects of Cr(VI) exposure in humans (Zhitkovich, 2005). Four of these studies documented increases among exposed groups compared to controls (Medeiros et al., 2003; Quievryn et al., 2001; Taioli et al., 1995; Costa et al., 1993). The fifth study did not document clear differences between exposed and controls but did identify positive associations between DNA-protein crosslinks and chromium in erythrocytes at low and medium exposure levels, with a saturation of crosslink incidence at higher levels (Zhitkovich et al., 1996a). Fifteen studies evaluated sister chromatid exchange (SCE). Elevated levels of SCEs following exposures are indicative of increased DNA repair and are considered biomarkers of exposure to potential genotoxic agents but do not correlate well with mutation frequency (Eastmond, 2014). Among these, six studies documented increased SCEs per cell among exposed groups of welders (Werfel et al., 1998) or electroplating workers (Wu et al., 2001; Wu et al., 2000; Lai et al., 1998; Deng et al., 1988; Stella et al., 1982) compared to control groups. Similarly, one study documented an association between urinary chromium and SCE (Sarto et al., 1982). Seven studies did not observe impacts on SCEs, either through comparing exposed and control groups (Benova et al., 2002; Nagaya, 1986; Koshi et al., 1984; Littorin et al., 1983; Husgafvel-Pursiainen et al., 1982) and/or through evaluating the association with urinary chromium directly (Nagaya et al., 1991; Nagaya et al., 1989; Nagaya, 1986). One study documented a decrease in SCE frequency among welders compared to controls, though the authors noted concerns with the alkaline filter elution that may have impacted the validity of the results (Popp et al., 1991).

Genetic polymorphisms

Genetic polymorphisms can alter individual susceptibility to health effects of environmental exposures, including chromium. Thirteen studies in humans were identified that evaluated genetic polymorphisms in relation to chromium exposure and cancer-related outcomes (mechanistic or apical). Seven studies evaluated genetic polymorphisms in relation to mechanistic outcomes relevant to cancer (e.g., mutations, genome instability). Of these, one focused on micronuclei, with interaction effects reported for some genes related to DNA repair and tumor suppression (XRCC3, BRCA2, NBS1) (Long et al., 2019). Two studies from the same lab group (Halasova et al., 2012; Halasova et al., 2008) reported increased chromosomal aberrations among welders with polymorphisms of one gene that encodes DNA repair enzymes (XRCC1) but not others (XPC, XPD, EPG, XRCC3, hOGG1). Similarly, polymorphisms in XRCC1 were also associated with increases in

DNA strand breaks among welders (Iarmarcovai et al., 2005) and measures of DNA damage such as olive tail moment, tail length, and tail DNA% among electroplating workers (Zhang et al., 2012). Finally, two studies of electroplating workers from another lab group evaluated potential differential effects on sister chromatid exchange due to polymorphisms in genes related to detoxification (GSTM1, GSTT1); interaction effects were detected for GSTT1 (Wu et al., 2001) in one study but not the other (Wu et al., 2000).

Four studies evaluated genetic polymorphisms in the context of cancer. One study identified an increased risk of lung cancer in individuals with certain polymorphisms in XPD (Sarlinova et al., 2015), which is involved in nucleotide excision repair. Three studies approached the question in a different way, probing the frequency of certain gene variants in cancer cases. Polymorphisms in the surfactant protein B gene were found to be more common in small-cell carcinomas from workers exposed to Cr(VI) compared to non-chromate-related small-cell carcinomas from matched controls (Ewis et al., 2006). In another study, the odds of hMLH1 polymorphisms was found to be elevated in chromate-related lung cancer cases compared to non-chromate-related hospital-matched controls (Halasova et al., 2016). Finally, one study evaluated microsatellite instability (operationalized as replication error (RER), defined as microsatellite instability at two or more loci) among individuals with lung cancer; study authors report increased frequency of RER among cases with chromate exposure compared to those without chromate exposure as well as an association between duration of chromate exposure and lung cancer cases with RER compared to those without RER (Hirose et al., 2002).

Although it is difficult to draw conclusions based solely on the human evidence, the existing data suggest that genetic polymorphisms may play a role in cancer susceptibility of individuals exposed to Cr(VI), and the impact of polymorphisms relevant to DNA damage and detoxification pathways in particular can provide important insight on the cancer MOA for Cr(VI).

Target tissue analyses of genotoxicity

A small number of studies conducting analyses of genotoxicity in human gastric fluid or primary human GI or lung cells were also identified. In a gastric reduction capacity experiment using pre- and post-meal gastric fluid samples from healthy volunteers (n = 8), higher reducing capacity and significantly decreased mutagenicity (evaluated by the Ames assay) were observed in post-meal samples compared to pre-meal samples. A 70% total Cr(VI) reduction was observed within 1 minute with a 98% reduction by 30 minutes (De Flora et al., 2016). Because gastric emptying occurs in vivo (reduction and emptying are competitive processes), a fraction of ingested Cr(VI) will empty to the small intestine prior to reduction (see Section 3.1 and Appendix C.1). In a study of lung reduction capacity by the same group, the S-9 fraction from pulmonary alveolar macrophages (PAM) isolated from the lung of human subjects (n = 47) was capable of lowering Cr(VI)-induced mutagenicity in the Ames assay by approximately 25% when preincubated for 1 hour prior to plating (Petrilli et al., 1986). Similar results were obtained by the S-12 fractions of peripheral lung parenchyma isolated from healthy subjects and from patients with lung cancer on

the mutagenicity of Cr(VI) in the Ames assay; samples from smokers had a significantly higher ability to reduce Cr(VI) (De Flora et al., 1987b).

Pool-Zobel et al. (1994) performed the comet assay for measuring DNA strand breaks on human mucosal cells from macroscopically healthy tissues of patients collected during biopsy treated with 0.087−0.349 μmoles/mL Cr(VI) in vitro. The results showed genotoxicity occurring at non-cytotoxic doses, with responses in the cells from humans paralleling those of cells from SD rats (see DNA damage section in synthesis of animal genotoxicity evidence). Similarly, a separate group reported statistically significant increases in DNA damage using the comet assay in two studies of human primary gastric mucosal cells exposed to concentrations ≥177 μM Cr(VI), which underwent repair within an hour (Trzeciak et al., 2000; Błasiak et al., 1999).

Tumor genotyping

The study of mutations occurring in oncogenes or tumor suppressor genes in tumor tissues can help identify chemical-specific driver mutations that could be key for tumor progression, as well as signature mutations that can potentially establish a causal association between chemical exposure and tumors. One study, Alguacil et al. (2003), evaluated mutations in the KRAS oncogene in tumor tissues, comparing pancreatic cancer cases with and without KRAS mutated tumors in individuals with inhalation exposure to chromium (ascertained using occupational history and a job-exposure matrix). The exposed workers with pancreatic tumors had increased odds of KRAS mutations in these tumors. Study authors also documented an increased proportion of G-to-T transversions with inhalation exposure to chromium. However, very few individuals were identified as having occupational chromium exposure, resulting in wide confidence intervals around the effect estimates (Alguacil et al., 2003). In addition, because pancreatic tumors have not been associated with occupational Cr(VI) exposure, and nearly 100% of pancreatic tumors (pancreatic ductal adenocarcinomas) have mutations in the KRAS gene (Waters and Der, 2018), this evidence may have little biological relevance to Cr(VI)-induced cancer.

Three studies evaluated p53 mutations among chromate factory workers with lung cancer, comparing cases with and without chromium exposure. Kondo et al. (1997) identified fewer p53 mutations among chromate workers. Yet, study authors also identified specific patterns of p53 mutations among lung cancer cases with prior chromate exposure, including double missense mutations. However, lack of adjustment for confounding and small sample size limit confidence in these findings (Kondo et al., 1997). Similarly, Katabami et al. (2000) detected an upregulation in cyclin D1 protein expression but no differences in p53 or bcl-2 protein expression in lung cancer tissues from chromate-exposed patients compared to non-exposed or pneumoconiosis lung cancer patients, though this study also had a small sample size and only considered confounding due to smoking status. Cyclin D1 is involved in the regulation of cell cycle progression and is elevated in a number of human cancers (Alao, 2007), and when paired with the absence of a protective p53-induced apoptotic response, may indicate a factor in Cr(VI)-induced cancer development. The third study, Halasova et al. (2010), determined that expression of the apoptosis inhibitor survivin protein

was decreased, concomitant with an increase in pro-apoptotic p53 levels, in former chromium workers with lung cancer compared to control lung cancer patients. However, little information was given regarding the potential exposures of these workers, and no information on confounders including smoking status was included. Although this finding is not surprising given these interconnected pathways of cell fate determination, the potential for co-exposures and co-morbidities precludes the ability to draw conclusions from these findings.

Overall, specific driver mutations or mutational signatures considered to be specific to Cr(VI) exposure have not been identified in exposed humans. However, there is evidence that critical human cancer effector pathways are directly and indirectly impacted after Cr(VI) exposure. Cr-DNA adducts, well established to occur in controlled conditions in cell cultures and acellular test systems in vitro (see Section 3.2.3.4 for a broader discussion of Cr-DNA adduct formation), could potentially provide additional support connecting exposure to genotoxic chemicals with effect. However, due to their transient nature, they do not appear to have the potential to be used as biomarkers of genotoxicity following Cr(VI) exposure in humans; accordingly, no evidence of the recovery of Cr-DNA adducts has been identified in Cr(VI)-exposed humans or animals.

Animal study evaluation summary

As described above in the introduction to the mutagenic MOA evaluation approach and in Appendix C.3.2.2, the available animal evidence prioritized as the most relevant for informing a mutagenic MOA analysis for cancer includes measures of gene mutation (prior to tumorigenesis), micronuclei induction, and chromosomal aberrations. These studies were prioritized for evaluation and synthesis in this section based on study design, namely if they were conducted in animals exposed via inhalation or intratracheal instillation, or via the oral route, including drinking water, diet, or gavage. Gavage and intratracheal instillation exposures were considered with the acknowledgment that these dosing regimens condense the exposure time while potentially inhibiting reduction kinetics leading to increased point-of-contact Cr(VI) exposure. Studies measuring DNA damage or indicators of DNA damage or using less relevant methods of chemical administration (i.e., i.p. injection) were not prioritized but are still considered as supplemental evidence to mutation and are summarized in the following section.

Table 3-18 summarizes the 11 animal studies of Cr(VI)-induced mutagenicity via inhalation or oral exposures (reporting 12 total endpoints) that were prioritized for evaluation. These consist of six studies measuring mutation frequency following short-term and subchronic exposures to drinking water (Aoki et al., 2019; Thompson et al., 2017; Thompson et al., 2015c; O'Brien et al., 2013; Kirpnick-Sobol et al., 2006) or via intratracheal instillation (Cheng et al. (2000; 1998); the preliminary and primary study results were reported in two separate publications), six studies measuring micronucleus incidence following acute, short-term, or chronic drinking water and/or gavage exposures (Thompson et al., 2015b; O'Brien et al., 2013; NTP, 2007; De Flora et al., 2006; Mirsalis et al., 1996; Shindo et al., 1989), and one dominant lethal test in rats exposed via intragastric instillation (Marat et al., 2018).

The endpoints specific to mutation, identified using the prioritization criteria for mutagenicity evidence relevant to cancer (outlined in Appendix C.3.2.2), were evaluated separately from any apical endpoints that may have also been reported in these animal bioassays (see Table 3-8). The majority of the prioritized studies are in vivo assays considered to be complementary, as the transgenic rodent assay primarily detects point mutations and small deletions (Dobrovolsky and Heflich, 2018), and the micronucleus assay can detect chromosomal aberrations and aneuploidy (Hayashi, 2016). Following study evaluation, all 12 studies of mutagenic endpoints were categorized as *low* confidence.

For many of the considered studies (Aoki et al., 2019; Thompson et al., 2017; Thompson et al., 2015c; Thompson et al., 2015b; O'Brien et al., 2013; NTP, 2007; De Flora et al., 2006; Mirsalis et al., 1996), the concern was not with the "quality" of the study, but rather with study designs that were not optimized for genotoxic endpoints and thus lacked sensitivity for detecting an effect if one were to be present, leading to deficiencies in the exposure sensitivity domain. According to the test guidelines (TG) adopted by the Organisation for Economic Cooperation and Development (OECD) for the transgenic rodent assay (TG 488, (OECD, 2020)) and the mammalian erythrocyte

micronucleus test (TG 474, (OECD, 2016a)), the two endpoints reported in most of the prioritized studies, these studies should include a range of doses with the top dose representing the maximum tolerated dose (MTD) that produces non-lethal toxicity in the animals (or, if not achievable, a daily dose of 1000 mg/kg-bw for a 28 day administration)[28]. This is to ensure the study is capable of characterizing the mutagenic potential of the chemical on the target tissue(s) by confirming the substance has reached the target tissue at levels high enough to induce toxicity, which is often the bone marrow for standard micronucleus tests in polychromatic erythrocytes. Testing for mutagenicity up to toxic levels is particularly important for increasing confidence in null findings in vivo for a substance known to be mutagenic in vitro, such as Cr(VI). The motivation for selecting a dose range to specifically study the induction of mutagenic effects at the same dose levels (albeit with shorter exposure durations) that caused preneoplastic lesions and tumors in these animals (e.g., up to 31.1 mg/kg-d Cr(VI) in female mice) is understandable. However, a bioassay properly designed to detect potential mutagenic effects from ingested Cr(VI)[29], a known carcinogen and a mutagen via other routes of exposure, was not identified.

Other concerns about the ability of these studies to appropriately characterize mutagenicity also contributed to their *low* confidence ratings. Deficiencies in the outcome sensitivity domain included studies that counted too few plaque-forming units in the transgenic rodent assay (Cheng et al. (2000; 1998)) or polychromatic erythrocytes in the micronucleus assay (O'Brien et al., 2013; Shindo et al., 1989), testing in tissues that did not develop tumors (Thompson et al., 2017), a mutation frequency background too high to reliably detect an effect (O'Brien et al., 2013), or failed positive controls (Thompson et al., 2015b). A few studies were deficient in results display sensitivity, including a failure to account for litter effects in a mutation study of exposures in mice in utero (Kirpnick-Sobol et al., 2006), not reporting the total number of cells scored for micronuclei (O'Brien et al., 2013), or pooling total micronuclei from multiple animals (Thompson et al., 2015b).

[28]TG 474 (OECD, 2016a): "The study should aim to identify the maximum tolerated dose (MTD), defined as the highest dose that will be tolerated without evidence of study-limiting toxicity, relative to the duration of the study period (for example, by inducing body weight depression or hematopoietic system cytotoxicity, but not death or evidence of pain, suffering or distress necessitating humane euthanasia. The highest dose may also be defined as a dose that produces toxicity in the bone marrow (e.g., a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood of more than 50%, but to not less than 20% of the control value)…If the test chemical does not produce toxicity in a range-finding study or based on existing data, the highest dose for an administration period of 14 days or more should be 1000 mg/kg body weight/day, or for administration periods of less than 14 days, 2000 mg/kg/body weight/day." TG 488 (OECD, 2020): "The top dose should be the Maximum Tolerated Dose (MTD). The MTD is defined as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality."

[29]There were issues with Cr(VI) palatability at high drinking water concentrations (above ~90 mg/L Cr(VI) in the NTP (2007) strain comparison study and at higher doses in the toxicity study), but in these cases it would also be acceptable to use gavage administration to confirm delivery of a sufficient dose of Cr(VI). Only one study included a gavage-administered dose that reached sufficient bone marrow toxicity, but this study was judged *low* confidence due to deficiencies in the reporting, confounding, and outcome sensitivity domains (Shindo et al., 1989).

One dominant lethal test identified did not report the strain of animals, test compound, or vehicle used (Marat et al., 2018). The prioritized studies are summarized in Table 3-19.

Table 3-18. Summary of prioritized animal studies for Cr(VI) mutagenicity and overall confidence classification [high (H), medium (M), low (L)] by outcome.
Click to see interactive data graphic for rating rationales.

				Mutagenic endpoints		
Author (year)	**Species (strain)**	**Exposure duration**	**Exposure route**	**Gene mutation**	**Micronuclei**	**Dominant Lethal test**
Cheng et al. (2000; 1998)	Mouse (C57BL/6 Big Blue® and nontransgenic C57BL/6), female	1, 2, or 4 wks post-instillation	Intratracheal instillation	L	–	–
Aoki et al. (2019)	Mouse (transgenic gpt delta), male	28 and 90 days	Drinking water	L	–	–
Kirpnick-Sobol et al. (2006)	Mouse (C57BL/6Jp^{un}/p^{un}), pregnant dams and offspring	GD 10.5–20.5	Drinking water	L	–	–
Thompson et al. (2017; 2015c)	Rat (transgenic Big Blue® TgF344), male	28 days	Drinking water	L	–	–
O'Brien et al. (2013) [related study: (Thompson et al., 2011)]	Mouse (B6C3F_1), female	90 days	Drinking water	L	L	–
NTP (2007)	Mouse (B6C3F_1), male and female; Mouse (B6C3F_1, BALB/c, *am3*-C57BL/6), male	90 days	Drinking water	–	L	–
Thompson et al. (2015b)	Mouse (B6C3F_1), female	7 days	Drinking water	–	L	–
De Flora et al. (2006)	Mouse (BDF$_1$), male and female; Mouse (Swiss albino) pregnant dams and fetuses	20 or 210 days or pregnancy duration	Drinking water, gavage, i.p.	–	L	–
Mirsalis et al. (1996)	Mouse (Swiss-Webster), male and female	2 days	Drinking water, gavage	–	L	–
Shindo et al. (1989)	Mouse (MS/Ae and CD-1), male	Bolus dose (acute)	Gavage, i.p.	–	L	–
Marat et al. (2018)	Rat ("mature white outbred"), male	60 days	Intragastric administration	–	–	L

Synthesis of animal genotoxicity evidence

The studies prioritized for being most informative for a mutagenic MOA analysis are summarized in Table 3-19.

Table 3-19. Prioritized genotoxicity studies in animals exposed to Cr(VI)

Reference	System/ Exposure	Endpoint/Results[a]	Comments
Tests in lung tissue			
Cheng et al. (2000; 1998) *Low* confidence	Mouse, transgenic C57BL/6 Big Blue®, female Intratracheal instillation (single administration): 0, 1.7, 3.4, or 6.8 mg/kg Cr(VI) Measured mutation frequency in lung at 1, 2, or 4 weeks post-exposure	Significantly increased mutation frequency at all doses; increased with dose and duration post-treatment Mutation spectrum: increased frequency of G:C to T:A transversions, associated with oxidative damage	Preliminary experiment identified doses >6.75 mg/kg were lethal Potentially underpowered with 4 mice per dose group Positive control not concurrently tested with Cr(VI)-treated group Inconsistent/low numbers of PFUs scored per animal Spontaneous mutations primarily G:C to A:T transitions
Tests in GI tissue			
Aoki et al. (2019) *Low* confidence	Mouse, transgenic gpt delta, male Drinking water, 28 d: 0, 30, or 90 mg/L Cr(VI) (0, 13, or 30 mg/kg-d Cr(VI)) Drinking water, 90 d: 0, 3, 10, or 30 mg/L Cr(VI) (0, 1.6, 6, or 17 mg/kg-d Cr(VI)) Measured mutation frequency in duodenum at 28 and 90 days	In mouse duodenum: No increased mutation frequency (gpt delta locus) relative to control at 28 or 90 d Mutation spectrum: slightly increased A:T to T:A transversions at 28 d but not at 90 d (significance unknown)	Study selected doses based on NTP 2-yr bioassay and did not include a top MTD, potentially biasing toward the null Positive control not concurrently tested with Cr(VI)-treated groups 90-d study potentially underpowered with 4 mice per dose group Spontaneous mutations primarily G:C to A:T transitions Positive control potassium bromate (but not Cr(VI)) had increased G:C to T:A transversions, associated with oxidative damage

Reference	System/ Exposure	Endpoint/Results[a]	Comments
Thompson et al. (2015c) *Low* confidence	Rat, transgenic Big Blue((R)) TgF344, male Drinking water: 180 mg/L Cr(VI), 28 d	In oral mucosa (upper inner gingiva and adjacent palate tissue and the upper outer gingiva and adjacent buccal tissue): No increase in mutation frequency (cII gene) relative to control	Study used single dose group based on NTP 2-yr bioassay top dose and did not include a top MTD, potentially biasing toward the null Cr levels in the gingival/ buccal and gingival/palate regions were 0.66 and 1.0 µg/g, respectively, compared to untreated Tg344 rats, which were 0.17 and 0.33 µg/g respectively in the gingival/buccal and gingival/palate regions Authors reported in vitro results showing enriched responses for p53, cell proliferation and apoptosis
Thompson et al. (2017) *Low* confidence		In duodenum: No increase in mutation frequency (cII gene) relative to control	Study used single dose group based on NTP 2-yr bioassay top dose and did not include a top MTD, potentially biasing toward the null Positive control not concurrently tested with Cr(VI)-treated group Rat small intestine is not a tumor target tissue
O'Brien et al. (2013) *Low* confidence	Mouse, B6C3F1, female Drinking water: 0, 0.1, 1.4, 4.9, 20.9, 59.3, and 181 mg/L Cr(VI) (0, 0.024, 0.32, 1.1, 4.6, 11.6, or 31.1 mg/kg-d Cr(VI)) 7 or 90 d (Continued analysis of tissues from Thompson et al. (2011))	Micronucleus assay, in crypt and villous cells from scraped duodenal epithelium: No increase in micronucleus frequency in crypt cells Statistically significantly increased micronuclei in villous cells from animals exposed to 11.6 mg/kg-d Cr(VI) for 90 days or 31.1 mg/kg-d Cr(VI) for 7 or 90 days ACB-PCR, in scraped duodenal epithelium: No induction of GGT to GAT mutations in KRAS codon 12 detected by ACB-PCR relative to control	Micronucleus assay: No baseline incidence of micronuclei established in these tissues Crypt cell data pooled from all animals per dose group and large variation in total cells counted per dose Total number of villous cells analyzed not presented ACB-PCR: High background mutant frequency Both endpoints: Study selected doses based on NTP 2-yr bioassay and did not include a top MTD, potentially biasing toward the null Positive control not concurrently tested with Cr(VI)-treated group

Reference	System/ Exposure	Endpoint/Results[a]	Comments
Thompson et al. (2015b) *Low* confidence	Mouse, B6C3F1, female Drinking water: 0, 1.4, 20.9, and 180 mg/L Cr(VI) (0, 0.32, 4.6, and 31.1 mg/kg-d Cr(VI)) 7 d	In duodenal crypts (villi not reported): No increase in micronucleus frequency relative to control No effect on levels of γH2AX	Study selected doses based on NTP 2-yr bioassay and did not include a top MTD, potentially biasing toward the null No baseline MN incidence established for these tissues, positive control DMH was null, number of cells analyzed inadequate to measure an effect 21 and 180 mg/L Cr(VI) significantly increased the number of crypt enterocytes, although no increase in crypt mitotic activity was detected No aberrant crypt or villous foci; no apoptosis in crypt cells
Tests in other tissues			
Kirpnick-Sobol et al. (2006) *Low* confidence	Mouse, C57BL/ 6Jp^{un}/p^{un}, female Drinking water: 0, 22, or 44 mg/L Cr(VI) at 10.5 to 20.5 days postcoitum (average dose of 4.4 or 8.8 mg/kg-day)	In 20-day-old offspring harvested to visualize eyespots corresponding to DNA deletions in their retinal pigment epithelium (RPE): Increased deletions with dose ($p < 0.01$)	Failed to account for litter effects, potentially biasing away from the null No information on blinding; concerning for this type of assay that requires manual counting of eyespots Positive control not concurrently tested with Cr(VI)-treated group No signs of toxicity observed
Marat et al. (2018) *Low* confidence	Rat, white outbred males Intragastric administration, 1 mg Cr/kg body mass, single dose, 60 days prior to mating with virgin female rats	Survival of F1 fetuses from F0 males exposed to Cr(VI): Ratio of live fetuses in the Cr(VI) treatment group compared to the control group = 0.665 indicating increased dominant lethal mutation frequency in exposed male rats	Deficiencies in reporting and information on lab proficiency/reproducibility Study also reported increased micronucleus frequency in bone marrow in rats exposed to a single i.p. dose of K2Cr2O7
NTP (2007) *Low* confidence	Study 1: Mouse, B6C3F$_1$ (5/sex/group) Drinking water: 0, 21.8, 43.6, 87.2, 174.5, or 350 mg/L Cr(VI), 90 d NTP estimated daily doses at 0, 3.1, 5.2, 9.1, 15.7, or 27.9 mg Cr(VI)/kg	In peripheral blood: B6C3F$_1$: No effect on %MN NCEs (males: $p = 0.857$; females: $p = 0.158$)	The reduction of PCE/NCE ratio in treatment groups was slight, indicating mild bone marrow toxicity, though this did not increase with dose

Reference	System/ Exposure	Endpoint/Results[a]	Comments
	Study 2: Mouse, B6C3F$_1$ (5/group), BALB/c (5/group), and *am3*-C57BL/6 (10/group), males Drinking water: 0, 21.8, 43.6, or 87.2 mg/L Cr(VI), 90 d NTP estimated average daily doses at 0, 2.8, 5.2, or 8.7 mg Cr(VI)/kg	In peripheral blood: B6C3F$_1$: NTP determined this result to be equivocal due to a trend test p-value very nearly significant (p = 0.031; α level = 0.025) and a significant response (p = 0.0193) in the highest dose group of 87.2 mg/L. BALB/c: No effect on %MN NCEs (p = 0.680) *am3*-C57BL/6: ↑ %MN NCEs (p < 0.001)	No effect on PCE/NCE ratio and no clinical signs of toxicity observed; failure to include an MTD potentially biases toward the null *am3*-C57BL/6 transgenic mice intended to measure mutation frequency, but technical difficulties prevented completion of this study
Mirsalis et al. (1996) *Low* confidence	Mouse, Swiss-Webster, M&F (5/sex/group) Drinking water: 0, 1, 5, or 20 mg/L Cr(VI), 48 h Gavage: 20 mL/kg of 0, 1, 5, or 20 mg/L Cr(VI), 2 doses, 24 and 48 h	In bone marrow: No effect on %MN PCEs	Study did not include enough information to accurately calculate a dose for either experiment Study did not include a top MTD (no effect on PCE/NCE ratio) potentially biasing toward the null
De Flora et al. (2006) *Low* confidence	Experiment 1: Mouse, BDF$_1$ males Drinking water: 0, 10, or 20 mg/L Cr(VI), 20 d Daily intake estimated at 3 and 6 mg/kg-bw for 10 and 20 mg/L, respectively Gavage or i.p.: 0 or 17.7 mg/kg Cr(VI), single dose, 24 h	Drinking water, in peripheral blood, day 0, 5, 12, and 20: no effect on %MN NCEs Drinking water, in bone marrow, day 20: no effect on %MN PCEs Gavage, in bone marrow, 24 h: no effect on %MN PCEs i.p. injection, in bone marrow, 24 h: significant increase in %MN PCEs (p < 0.001)	Results of %MN NCEs at day 5–20 are uninterpretable; evaluation of MN in mature erythrocytes requires 4 weeks of continuous treatment (Macgregor et al., 1990) Per os exposure groups did not include a top MTD (no effect on PCE/NCE ratio) potentially biasing toward the null
	Experiment 2: Mouse, BDF$_1$ M&F Drinking water: 0, 5, 50, and 500 mg/L Cr(VI), 210 d Daily intake estimates per dose group, respectively: Males: 1.65, 16.5, and 165 mg Cr(VI)/kg-bw Females: 1.4, 14, and 140 mg Cr(VI)/kg-bw	In peripheral blood, day 0, 14, 28, 56, and 147: no effect on %MN NCEs In bone marrow, day 210: no effect on %MN PCEs	Results of %MN NCEs at day 14 are uninterpretable; evaluation of MN in mature erythrocytes requires 4 weeks of continuous treatment Study did not include a top MTD (no effect on PCE/NCE ratio) potentially biasing toward the null Cr(VI) groups had similar drinking water consumption at all doses Slight decrease in body weight in Cr(VI)-treated animals, especially females

Reference	System/ Exposure	Endpoint/Results[a]	Comments
	Experiment 3: Mouse, pregnant Swiss albino Drinking water: 0, 5, or 10 mg/L Cr(VI) (as both sodium dichromate dihydrate (SDD) and potassium dichromate (PDC)) throughout pregnancy duration, 18 d i.p.: 0 or 17.7 mg/kg Cr(VI) (as both SDD and PDC), PD 17, 24 h	In the bone marrow of dams or in the liver or peripheral blood of fetuses: Drinking water: no effect on %MN PCEs i.p. exposures: micronuclei significantly increased in all tissues ($p < 0.001$)	Per os exposure groups did not include a top MTD (no effect on PCE/NCE ratio) potentially biasing toward the null No effect on fetus body weights
Shindo et al. (1989) *Low* confidence	Mouse, MS/Ae and CD-1, male Gavage and i.p. injection: 2.68, 5.36, 10.7, 21.4, 42.8, and 85.7 mg Cr(VI)/kg, bolus dose, 24 h	Gavage, in bone marrow: No effect on %MN PCEs up to acutely toxic oral gavage doses that reduced PCE/NCE ratio >50% i.p. injection, in bone marrow: Dose-dependent increase in %MN PCEs and decrease in PCE/NCE ratio	Calculated LD50s: MS/Ae mice LD50: 80.3 mg Cr(VI)/kg p.o., 13.4 mg Cr(VI)/kg i.p. CD-1 mice LD50: 48.2 mg Cr(VI)/kg p.o., 8.57 mg Cr(VI)/kg i.p. Study reported mean/SD per dose group but did not report the number of animals tested per group Baseline MN incidence extremely low

[a]Results reported in the same study of genotoxicity endpoints or exposure routes that did not meet PECO have also been included here for study context.

Gene mutations

Three studies in mice and rats were identified that used transgenic models to measure mutation frequency in tumor target tissues after short-term or subchronic exposures to Cr(VI) in drinking water (Aoki et al., 2019; Thompson et al., 2015c) or in the lung following intratracheal instillation (Cheng et al. (2000; 1998)). The rodents contain transgenes (i.e., reporter genes integrated into their genome) that can detect point mutations in any tissue studied. Cheng et al. (2000; 1998) exposed female transgenic C57BL/6 Big Blue® mice to Cr(VI) via intratracheal instillation, then measured the mutation frequency in the *lacI* transgene in lung tissues after 1, 2, or 4 weeks post-instillation. This study was found to be *low* confidence, primarily due to concerns regarding the number of animals per dose group (four; five is the current minimum recommendation (OECD, 2020)) and the low and inconsistent number of plaque-forming units evaluated, which were pooled per dose group and not reported per mouse. A preliminary study determined that doses ≤6.75 mg/kg were not lethal; the second experiment included dose groups exposed to 0, 6.8, 3.4, and 1.7 mg/kg Cr(VI). The study reported increasing mutation frequency with dose and time post-instillation; at the top dose after 4 weeks, the mutation frequency was 4.7-fold of background levels, although there is some concern that the mutation frequency in the vehicle

control providing comparison was only assessed at 1 week post-treatment. The observed increase of mutation frequency with time up to 4 weeks post-treatment corresponds to the average cell turnover time of 28 days in lung tissue.

In a study conducted by members of the same group that created the transgenic *gpt* delta mouse used in the study (Nohmi et al., 1996), Aoki et al. (2019) used male mice to examine mutation frequency in the duodenum after 28 or 90 days of exposure via drinking water, at concentrations of 0, 30, and 90 mg/L Cr(VI) (28 days) or 0, 3, 10, and 30 mg/L Cr(VI) (90 days). This group selected doses for both exposure periods based on the doses used in the NTP 2-year bioassay with the exception of the lowest dose selected [3 mg/L Cr(VI)], which was less than the lowest dose used by NTP [5 mg/L Cr(VI)]. No significant increase in mutation frequency was detected after either time period. Although this study was otherwise well-conducted, deficiencies in study design led to sensitivity concerns indicating potential for bias toward the null, leading to overall *low* confidence. Use of concurrently run positive controls and inclusion of a dose that induced clear clinical signs of toxicity would have increased confidence in the negative findings for this assay.

A transgenic 28-day Big Blue® TgF344 rat study conducted by Thompson et al. (2017; 2015c) reported exposure to 180 mg/L Cr(VI) in drinking water also did not significantly increase the mutant frequency in the gingival/buccal or gingival/palate regions in the oral cavity of rats or in the rat duodenum. Similar to Aoki et al. (2019), the selection of a single Cr(VI) exposure group that was not high enough to induce systemic toxicity in a short-term bioassay led to reduced confidence in the sensitivity of this study design to detect a positive result and an overall *low* confidence judgment. In addition, the inclusion of rat duodenal tissues in this mutation assay provides little value to mechanistic interpretation given the small intestine is not a tumor target tissue in rats.

In another *low* confidence mutation study by the same group, O'Brien et al. (2013) conducted an analysis of KRAS codon 12 GGT to GAT mutations in mice, which are associated with human colorectal cancer and metastasis (Jones et al., 2017; Margonis et al., 2015). The study used tissues obtained from a previous subchronic bioassay in female mice (Thompson et al., 2011). The detection method, allele-specific competitive blocker polymerase chain reaction (ACB-PCR), was developed and validated by one of the study authors (Mckinzie and Parsons, 2002) and is a sensitive method for detecting specific mutations. There were no statistically significant Cr(VI) treatment-related increases measured for KRAS codon 12 GAT mutations; however, results were difficult to interpret due to the lack of a concurrent positive control and the high background mutation incidence (10^{-2} to 10^{-3}) compared to previous findings of spontaneous mutation frequency in mouse lung [3.88×10^{-4}; (Meng et al., 2010)], rat distal colon [12.9×10^{-5}; (Mckinzie and Parsons, 2011)], or human colonic mucosa [1.44×10^{-4}; (Parsons et al., 2010)]. Although this was a 90-day study, the dose levels tested in drinking water were selected to replicate those used in the 2-year NTP bioassay [up to 180 mg/L Cr(VI)] and did not include a higher dose to determine

whether mutations would have been induced at toxic levels, reducing the sensitivity of this study to detect an effect.

In a mouse model for measuring mutant frequency, the C57BL/6J p^{un}/p^{un} mouse strain takes advantage of a naturally occurring mutation, a tandem duplication at the pink-eyed dilution (*p*) locus, that causes the mice to have pink eyes (Brilliant et al., 1991). Exposure to mutagens that induce deletions via homologous recombination during fetal development can lead to reversion of this unstable mutation back to black-pigmented cells, or eyespots, which are visible and quantifiable. Although this assay developed by Schiestl et al. (1997) has not become part of the standard testing battery for the detection of mutagens, it represents a highly sensitive assay for detecting deletion mutations in single cells that are caused by transplacental exposures during embryonic development. The Schiestl lab (Kirpnick-Sobol et al., 2006) exposed female C57BL/6J p^{un}/p^{un} mice to 22 or 44 mg/L Cr(VI) in drinking water from 10.5 to 20.5 days post-coitum. Despite a somewhat elevated background frequency ($\sim 10^{-4}$), dose-dependent, statistically significant increases in mutations were observed in offspring ($p < 0.01$). However, the results of this study were presented as the mean of individual pups without taking litter effects into account, potentially overestimating the statistical significance of experimental findings (Haseman et al., 2001) and leading to bias away from the null. Therefore, this study was judged to be *low* confidence for this outcome.

One rodent dominant lethal test was identified (Marat et al., 2018). This assay detects gene and/or chromosomal mutations produced in male germ cells during a pre-mating exposure period, causing fetal death (OECD, 2016b). Marat et al. (2018) reported a dominant lethal mutation frequency of 0.665 by comparing the number of live F1 fetuses to control after exposure of F0 male rats to 0.353 mg/kg-day Cr(VI) by oral gavage, with increases in pre- and post-implantation mortality. The dominant lethal test appears to have been conducted appropriately and detected a 10-fold increase in post-implantation mortality, but this study was found to be *low* confidence due primarily to reporting deficiencies.

Micronuclei

Mutation studies can also measure increased incidences of heritable genetic alterations due to numerical or structural changes in the chromosomes of animals exposed to Cr(VI) in vivo. Four studies measuring changes in micronucleus frequency in the peripheral blood or bone marrow of mice exposed to Cr(VI) via drinking water or oral gavage were identified. In the 90-day bioassay conducted by NTP (2007), two micronucleus assays were conducted in mice exposed to Cr(VI) in drinking water. Study 1 exposed $B6C3F_1$ male and female mice up to 350 mg/L Cr(VI), and Study 2 exposed male $B6C3F_1$, BALB/c, and *am3*-C57BL/6 mice up to 87.2 mg/L Cr(VI). $B6C3F_1$ mice did not have increased frequencies of micronuclei in Study 1, but in Study 2, the result was considered equivocal due to a nearly statistically significant increased trend ($p = 0.031$; the one-tailed trend test required a $p < 0.025$ for significance). For the two other strains tested in Study 2, BALB/c mice also showed no increase in micronucleus frequency, but the top two dose groups of *am3*-C57BL/6

mice had statistically significant increases in micronuclei (p = 0.0025 and 0.0001 at 43.6 and 87.2 mg/L, respectively), as well as a statistically significant trend (p < 0.001), with no evidence of bone marrow toxicity. Although 5 animals per dose group is the minimum required for this test, it is of note that the micronucleus test with the only clear, statistically significant positive result reported by NTP (2007), in *am3*-C57BL/6 mice, tested twice as many animals (10/dose group), increasing the power of this study to detect an effect. This transgenic strain of mice was specifically included to perform an analysis of mutation frequency that was unsuccessful due to technical difficulties; however, there is no reason to suspect that the endogenous genome of transgenic mice would be unusually sensitive to clastogenic or aneugenic damage, and no data exist to suggest strain-specific susceptibility.

The interpretation of negative results for the hazard identification of micronucleus incidence in erythrocytes requires confirmation that the test agent reached the bone marrow at a sufficient dose to induce erythropoietic toxicity; the OECD Test Guidelines (OECD, 2016a) recommend that the highest dose should reduce the percentage of polychromatic erythrocytes (PCEs, also known as reticulocytes) among total erythrocytes (normochromatic erythrocytes, or NCEs) by at least 50%. In Study 1, a slight decrease in %PCEs among total NCEs was noted, indicative of toxicity in the bone marrow, but this reduction was relatively small (19% and 25% reduction compared to controls in male and female mice, respectively, at 350 mg/L) and did not increase with dose. The mice in Study 2, exposed to lower concentrations of Cr(VI), had no decreases in %PCEs. However, the top doses from each study caused reductions in body weight gain (which the study authors attributed to decreased palatability causing reduced food intake and not to Cr(VI)-induced toxicity) indicating that higher doses could not have been administered in drinking water. The NTP study, a well-conducted bioassay, was *high* confidence for the histopathological measures, but for the reasons described above was found to be *low* confidence for this endpoint. Although some toxicity was measured in the bone marrow in one (of two) arm of the study, a study design including more animals and higher doses, perhaps administered via gavage to avoid palatability issues, would have increased the sensitivity of this study to detect a positive result and/or increased confidence in the negative/equivocal findings.

Two otherwise well-conducted in vivo micronucleus studies were found to be *low* confidence for sensitivity concerns. Mirsalis et al. (1996) dosed mice via drinking water and gavage up to 20 mg/L Cr(VI) for 48 hours and did not detect an increase in micronucleus frequency or any effect on PCE/NCE ratio in the bone marrow. In another large study using far higher doses for a longer duration, De Flora et al. (2006) exposed mice to up to 500 mg/L in drinking water for 210 days in addition to exposures to pregnant dams of 10 mg/L in drinking water for the duration of pregnancy. However, no increased incidence of micronuclei or effect on PCE/NCE ratio was observed in the peripheral blood or bone marrow of exposed adults or in the liver or blood of fetuses exposed in utero. In another branch of this study, De Flora et al. (2006) also dosed mice with single i.p. injections of 17.7 mg/kg-bw Cr(VI), which produced positive results for

micronucleus induction; these subtoxic exposures were considered positive controls for the route comparison study, emphasizing the importance of pharmacokinetic considerations for Cr(VI) exposures. This study also screened NCEs from peripheral blood for micronuclei after 10 or 20 mg/L drinking water exposures for 20 days but these data are not considered (i.e., *uninformative*) as this exposure duration is insufficient for detecting micronuclei in mature erythrocytes (Macgregor et al., 1990).

One study, Shindo et al. (1989), did include a top dose (85.7 mg Cr(VI)/kg) that reached sufficient bone marrow toxicity. This well-conducted study was part of a larger effort by the Collaborative Study Group for the Micronucleus Test to establish best practices for this assay. The group conducted a pilot test to determine LD50s for each strain and route (oral and i.p.). A micronucleus test was then conducted, finding no increases in micronucleus frequency from acute oral exposures that reached a maximum tolerated dose in each strain. This study, however, was determined to be *low* confidence due to lack of reporting the number of animals tested and not establishing a sufficient background level of micronucleated PCEs to ensure adequate detection sensitivity in the study; for the CD-1 mice, the background micronucleus frequency was zero.

While the micronucleus assay has been traditionally performed in peripheral blood or bone marrow, it has been developed for use in other tissues provided the test is optimized for sensitivity (e.g., ensuring the test captures cells during the first cell division post-exposure). Notably, some GI tract mutagens [e.g., N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-methyl-N-nitrosourethane (NMUT)], do not show increased micronucleus frequency in the peripheral blood or bone marrow due to pharmacokinetic considerations, and adapting the MN assay for use in the GI tract, where the cellular turnover rate is 3–5 days, has yielded positive results for GI carcinogens known to be mutagenic (e.g., Okada et al. (2019)). Only two studies, conducted by the same group, were identified that specifically measured micronuclei in duodenal epithelial cells of mice exposed to Cr(VI) in drinking water (Thompson et al., 2015b; O'Brien et al., 2013).

O'Brien et al. (2013) identified micronuclei as well as mitotic and apoptotic cells in fully intact crypts from formalin-fixed and paraffin-embedded duodenal tissues obtained from a previous subchronic bioassay in female mice (Thompson et al., 2011). Because the bioassay had been conducted previously, appropriate positive controls were not run concurrently, which would be useful for establishing proficiency in this less standardized tissue for this assay (compared to the bone marrow or peripheral blood) for which no historical control data is available. In crypt cells, zero micronuclei were reported for every dose group; this, and the lack of cytotoxicity detected in these tissues even at the top dose (as measured by mitotic indices), indicate that the study was also likely not sensitive enough to detect an effect in these tissues, leading to a judgment of *low* confidence. At a minimum, scoring enough cells to detect a background rate for micronuclei incidence would have helped increase confidence in these findings. In the villous cells, however, statistically significantly increased numbers of cells with micronuclei were observed at the top dose at day 7 and the two highest dose groups (60 and 180 mg/L Cr(VI)) at day 90. The micronuclei

counts were pooled per dose group, and the total number of cells scored was not reported, so frequency cannot be determined, contributing to the *low* confidence judgment for this endpoint.

In the second micronucleus study in the GI tract by this group, Thompson et al. (2015b) again reported no increased micronuclei in duodenal crypt cells, but this study did not report whether there were again increased micronuclei in villous cells. Concerns regarding the sensitivity of the study design primarily involve the lack of establishing proficiency in this nonstandard assay. Specifically, again, a baseline number of micronucleated cells in crypts and/or duodenal enterocytes was not established; two exposure groups [180 mg/L Cr(VI), and the positive control, 65 mg/kg DMH] reported zero micronuclei in 5161 and 3153 cells, respectively. These groups had lower numbers of cells analyzed than the vehicle control, which screened 6694 cells to identify four micronucleated enterocytes (0.06%). Therefore, sufficient numbers of cells should have been counted for all dose groups to increase confidence in the sensitivity of this assay to detect reliable negative result. In addition, the top dose did not induce a change in mitotic indices in the crypts which was interpreted as a lack of cytotoxicity, indicating a lack of sensitivity for this endpoint (see above discussion on sensitivity concerns for this assay).

Of primary concern regarding the sensitivity of Thompson et al. (2015b) is the lack of micronuclei detection or other nuclear damage in animals dosed with 65 mg/kg DMH via gavage, or the low, nonsignificant levels of micronuclei reported for i.p. injection of DMH. DMH (1,2-dimethylhydrazine) is a colon carcinogen and alkylating agent widely used to induce colon tumors in animal models (Vanhauwaert et al., 2001) and has been used as a positive control to validate the micronucleus assay in the GI tract by other groups (Coffing et al., 2011; Ohyama et al., 2002; Goldberg et al., 1983). When administered via gavage or i.p., it induces increased micronucleus frequency in the mouse colon (Ohyama et al., 2002; Vanhauwaert et al., 2001; Goldberg et al., 1983). Another study validating the micronucleus assay in GI tissues dosed mice with DMH via gavage at 16.5, 33, 50, and 66 mg/kg and reported statistically significant, dose-dependent increases in micronuclei in the duodenum and colon at all doses tested (Coffing et al., 2011), with micronuclei detected at a higher frequency in the duodenum than in the colon. Therefore, this study was judged to be *low* confidence for this endpoint.

Supporting genotoxicity evidence

DNA damage

Genotoxicity endpoints that did not meet the mutagenicity prioritization criteria have also been reported in animal studies. These include measures of DNA damage that may not reflect actual mutation frequency, as well as studies using less relevant routes of exposure (i.e., i.p. injection studies). The prioritization strategy for identifying these studies and study details is summarized in Appendix C.3.2.2.

Only one animal study was identified that reported DNA damage measures following direct exposure to the lung. Gao et al. (1992) exposed Wistar rats to 0.45 and 0.87 mg/kg Cr(VI) via intratracheal instillation and detected a significant increase of DNA strand breaks in peripheral

lymphocytes after 24 hours. Several drinking water exposure studies were identified that reported mostly negative findings for DNA damage. Thompson et al. (2015b; 2015a) conducted immunohistochemical staining for phosphorylated histone H2AX (γH2AX), a marker of DNA double-strand breaks, in the intestinal villi and crypts of mice after oral exposure. Immunohistochemical grading reported moderate staining in the crypts that was not treatment-related, and moderate staining in the villi after exposure to 31 mg/kg Cr(VI)-day (high dose) after 13 weeks (Thompson et al., 2015a). A 7-day follow-up study by the same group also reported no treatment-related increase in γH2AX foci in the crypts, although these results may have biased toward the null due to the 24 hour recovery period given the potentially rapid disappearance of γH2AX (Thompson et al., 2015b). Another group reported a 1.5-fold increase in γH2AX in the 'distal section' of the GI tract in C57BL/6J mice exposed to up to 1.9 mg/L Cr(VI) in drinking water for 150 days, although the low number of animals studied (2/group) make these findings less informative (Sánchez-Martín et al., 2015). A separate genotoxicity study reported no evidence of DNA-protein crosslinks in GI tissues (forestomach, glandular stomach, and duodenum) of female SKH-1 mice after 9 months of low dose oral exposure to 1.20 and 4.82 mg Cr(VI)/kg-day through drinking water (De Flora et al., 2008).

Three studies in mice administering Cr(VI) via gavage reported significant, dose-dependent increases in DNA damage, measured by the comet assay, in multiple tissues, including lymphocytes (Wang et al., 2006), leukocytes (Dana Devi et al., 2001), stomach, colon, liver, kidney, bladder, lung, and brain (Sekihashi et al., 2001). Single, bolus gavage doses greatly condense the exposure time, inhibiting gastric reduction (ad libitum drinking water exposures are distributed over a 24-hour period, whereas gavage occurs over a very short period). This difference in pharmacokinetics could potentially explain the difference in genotoxicity results between gavage and drinking water observations. The only tissue Sekihashi et al. (2001) tested that did not find an increase in DNA damage was the bone marrow, and no indications of cytotoxicity were observed in the animals, indicating that Cr(VI) did not reach the bone marrow at sufficient concentrations to induce DNA damage (Dana Devi et al., 2001; Sekihashi et al., 2001).

Similarly, studies in rats and mice uniformly indicate Cr(VI) can cause gene and chromosomal mutations and DNA damage when injected intraperitoneally (i.p.); these are summarized in Appendix Table C-52. While less informative for GI tract cancers, intraperitoneal dosing experiments are considered supplemental to oral dosing studies in providing mechanistic evidence to inform mutagenic and genotoxic effects. Dosing via i.p. injection results in higher systemic tissue concentrations of Cr(VI) compared to oral and inhalation exposure because this route bypasses Cr(VI) reduction mechanisms that would otherwise dampen systemic Cr(VI) distribution and absorption (see Section 3.1 and Appendix C.1). Systemic effects are more likely following i.p. injection compared to oral exposure. However, some mechanistic studies aim to examine the effects of Cr(VI) on target tissues, irrespective of route, and i.p. injections may be the

only feasible method to expose some systemic target organs to carefully controlled and consistent concentrations of Cr(VI).

Although in vitro studies of human cells were prioritized over other mammalian cells, Pool-Zobel et al. (1994) compared responses from both human and rat cells. This study performed the comet assay for measuring DNA strand breaks on human and rat gastric mucosal cells from macroscopically healthy tissues of patients collected during biopsy or from Sprague-Dawley rats treated with 0.087−0.349 μmoles/mL Cr(VI) in vitro. The results showed genotoxicity occurring at non-cytotoxic doses, with responses in the cells from SD rats paralleling those from human cells, providing some evidence of species concordance for genotoxicity induced by Cr(VI).

Signature mutations

Other investigations of specific Cr(VI)-induced mutations that may be relevant to GI carcinogenesis have been reported. An analysis of the specific types of point mutations induced by a chemical can determine whether, compared to spontaneous mutations, certain mutations are more associated with exposures, i.e., signature mutations. Chemical-specific mutational signatures can potentially establish an association between chemical exposure and mutation, as well as lending mechanistic insight to the types of DNA damage most associated with the specific mutation. In addition to analyzing mutation frequency, two studies examined specific types of point mutations in the mouse small intestine after 28 or 90 days of exposure. G:C to T:A transversions, mutations that frequently result from the DNA damage associated with oxidative stress, were observed to occur at a slightly higher frequency (11%) in the lung of the Cr(VI)-treated transgenic mice (6.75 mg/kg, intratracheal instillation) (Cheng et al. (2000; 1998)), consistent with in vitro findings by this group (Liu et al., 1999). The G:C to T:A transversions correlated with glutathione levels, presumably because the antioxidant is reducing higher levels of intracellular Cr(VI) and thus increasing reactive oxygen species generation.

In another study in transgenic mice, an increase in G:C to T:A transversions was not observed in mutations recovered from the duodenum in animals exposed to Cr(VI) in drinking water (Aoki et al., 2019). This study did, however, detect a higher rate of A:T to T:A transversions in the Cr(VI)-exposed animals at 28 days that was not detectable at 90 days; the significance of this mutation in relation to Cr(VI) is not known, but it indicates a potential signature mutation that could be investigated further. The Cheng et al. (2000; 1998) study reported a higher frequency of all mutation types in Cr(VI)-exposed animal lung tissue compared to controls, whereas the Aoki et al. (2019) study did not detect an increase in mutations over background in the duodenum. Although the study did not conduct additional testing to determine whether this difference is attributable to a lack of oxidative DNA damage (and subsequent G:C to T:A transversions) in the animals in the Aoki et al. (2019) study, it is possible that mutations related to oxidative damage are more likely to be induced in a single high intratracheal instillation exposure (6.75 mg/kg Cr(VI)) in Cheng et al. (2000; 1998), compared to a longer, lower dose exposure period (up to 0.7 mg/kg-d for 28 days or 0.45 mg/kg-d for 90 days, drinking water) used by Aoki et al. (2019). Some consistency in results is

noted by the finding that both studies reported that a high proportion of spontaneous mutations were G:C to A:T transitions. Overall, there is not enough evidence to conclude that there may be a signature mutation associated with Cr(VI) exposure.

Integration of genotoxicity evidence

Cr(VI) has been shown to be genotoxic and induce mutations in vitro and in animals exposed via i.p. injection (summarized in Appendix C.3.3.3 and C.3.3.4), providing mechanistic support for the mutagenicity of Cr(VI) in these specific exposure scenarios. The evidence is less clear from in vivo exposures, where pharmacokinetics can influence the ability and extent of Cr(VI) reaching the tissues at concentrations capable of inducing detectable mutations. Therefore, genotoxicity studies were prioritized to identify gene and chromosomal mutation studies in vivo using inhalation and oral routes of exposure more relevant to humans.

Occupational exposure studies provide the most human relevant information for mutagenic risk from Cr(VI) exposures. Consistent evidence of the mutagenic and genotoxic effects associated with Cr(VI) exposure is provided by human studies across a diversity of study populations and industrial settings (summarized in Table 3-17 and Appendix Table C-47). In studies detecting transmissible genetic damage (i.e., micronuclei and chromosomal aberrations), increased micronucleus frequency and, to a lesser extent, chromosomal aberrations were consistently detected in the peripheral blood lymphocytes and exfoliated nasal and buccal epithelial cells of exposed workers. These biomarkers have been shown to be positively associated with an increased risk of cancer in humans (Bonassi et al. (2011b; 2008; 2007), (Norppa et al., 2006)). The data for micronuclei and chromosomal aberrations are supported by additional evidence of genotoxic responses to Cr(VI) exposure in humans, including DNA strand breaks, adducts, and crosslinks (summarized in Appendix Table C-49).

No studies investigating genotoxicity in nonneoplastic lung tissues were identified in the occupational exposure studies, but there was consistent evidence of increased micronucleus frequency in buccal cells from workers occupationally exposed to Cr(VI) via chrome plating and welding from two *medium* confidence studies (El Safty et al., 2018; Sudha et al., 2011) supported by findings reported in three *low* confidence studies (Qayyum et al., 2012; Danadevi et al., 2004; Benova et al., 2002). Although occupational exposure occurs primarily via inhalation, changes in buccal cells can serve as a surrogate of direct Cr(VI) exposures to the GI tract in humans if ingested Cr(VI) is able to reach those tissues in comparable amounts. Micronucleus frequency in these workers was found to correlate with blood chromium levels (El Safty et al., 2018; Qayyum et al., 2012; Danadevi et al., 2004), with work duration (Danadevi et al., 2004), and with systemic measures of DNA damage (e.g., 8-OHdG adducts, DNA strand breaks) (El Safty et al., 2018; Sudha et al., 2011; Danadevi et al., 2004).

The experimental evidence base of gene and chromosomal mutation studies in animals is smaller and composed entirely of *low* confidence studies (see Appendix Figures C-22 to C-25 for a

visual comparison of the reported findings from the oral exposure studies). One study was identified that exposed animal lung tissues directly to Cr(VI) (via intratracheal instillation) and reported dose-dependent increases in mutation frequency that increased with time from 1 to 4 weeks post-exposure (Cheng et al. (2000; 1998)). Although this is only one *low* confidence study, it is coherent with the findings in exposed humans and demonstrates the mutagenicity of Cr(VI) when it comes into direct contact with tissues.

A slightly higher number of studies investigating mutagenicity via the oral route are available. Four drinking water and/or gavage studies in mice measured micronucleus frequency in the peripheral blood or bone marrow, the tissues most commonly studied in the micronucleus assay due to the requirement of exposing actively dividing cells. Acute and subchronic studies by NTP found mixed results among three strains of mice (NTP, 2007), while three additional studies reported negative results in the bone marrow and/or peripheral blood (De Flora et al., 2006; Mirsalis et al., 1996; Shindo et al., 1989). When interpreting genotoxicity results, particularly negative results for a substance known to be mutagenic in other exposure scenarios, it is important to confirm that the test substance reached the tissues tested. In vivo micronucleus assays are designed to inform decisions regarding the mutagenic potential of a chemical (Eastmond et al., 2009), but if the doses selected for testing are lower than levels inducing some toxicity in the target tissues, it is not possible to conclude the chemical would not be a mutagen at higher, subtoxic or even toxic doses. For some of these studies, there is reason to suspect the exposures were not high enough to achieve adequate tissue concentrations in the bone marrow. For example, although pharmacokinetic findings by NTP (2007) indicate that Cr(VI) can reach the bone (or femur) at concentrations above 10 mg/L Cr(VI) (approximately 1–2 mg/kg-d), two of these studies exposing animals to concentrations up to 20 mg/L Cr(VI) in drinking water (De Flora et al., 2006; Mirsalis et al., 1996) did not detect increases in micronuclei, and also did not detect decreases in the PCE/NCE ratio, which would indicate toxicity in the bone marrow as specified by standard guidance for this assay (OECD, 2016a). A third study, exposing animals via gavage to much higher doses (bolus dose, up to 86 mg/kg Cr(VI)[30]), also reported negative findings that were observed in animals with significant bone marrow toxicity, but this study was *low* confidence due to the lack of establishing a background spontaneous rate of micronucleus incidence and not reporting the number of animals tested (Shindo et al., 1989).

The subchronic bioassay by NTP exposed male and female B6C3F$_1$ mice to concentrations in drinking water up to 350 mg/L Cr(VI) and did not detect increases in micronucleus frequency; these animals had a slight induction of bone marrow toxicity, though decreased palatability in these animals prevented these investigators from achieving a higher tissue concentration, and led to the selection of lower doses for their second study (NTP, 2007). There were some positive findings in the second study, a mouse strain comparison of toxicity responses that dosed up to 87.2 mg/L

[30]As a comparison, drinking water exposure of the top concentration of 350 mg/L Cr(VI) in Study 1 by NTP (2007) yields a daily dose of approximately 20 mg/kg-d, which is distributed over a longer period of time.

Cr(VI), with *am3*-C57BL/6 positive, BALB/c negative, and B6C3F$_1$ nearly statistically significant (see Table 3-19). This was despite a complete lack of toxicity in the bone marrow in these animals. It is possible that, due to pharmacokinetic variability, Cr(VI) concentrations in drinking water do not always reach sufficient concentrations in the bone marrow to induce significant mutagenicity in that tissue, making this test in bone marrow tissues or cells a less sensitive measure for detecting mutagenic potential in GI tissues following drinking water exposures. To enter bone marrow, orally ingested Cr(VI) must escape 1) extracellular reduction in the GI tract lumen, 2) extracellular reduction or cellular uptake in the liver and portal blood, and 3) extracellular reduction or cellular uptake in systemic blood. Unlike gastrointestinal tract tissues which may be more directly exposed to higher sustained levels of Cr(VI), the bone marrow may receive lower levels of exposure.

Evidence in tumor target tissues, as with the mutation study in the lung, is considered more informative due to the point of contact uptake of Cr(VI) and intracellular reduction that initiates potential carcinogenic pathways associated with Cr(VI) exposure (see Section 3.2.3.4). Three studies directly investigated mutation frequency in tissues in the mouse duodenum or the rat oral cavity following drinking water exposures. Two are gene mutation studies that examined target tissues in the mouse duodenum (Aoki et al., 2019) or the rat oral cavity (Thompson et al., 2015c) of transgenic rodents following subchronic drinking water exposures. Neither of these *low* confidence studies observed significant increases in mutation frequencies. These studies designed the dosing regimen based on the NTP 2-year bioassay and did not cover a range of doses that included a toxic dose, which would have increased confidence in this study's ability to detect an effect.

A third study, O'Brien et al. (2013), did not detect an increase in KRAS codon 12 GGT to GAT mutations in the mouse duodenal tissues. While KRAS mutations, primarily occurring in codons 12 and 13, have been identified in 35–45% of human colorectal cancers (Nguyen and Duong, 2018), and many types of codon 12 mutations have been identified in tumors of the GI tract in humans (Peng and Zhao, 2014), there are no data to establish the presence of codon 12 GGT to GAT mutations in tumors from Cr(VI)-exposed workers, or in oral rat or duodenal mouse tumors induced by Cr(VI). Furthermore, a comparison study with spontaneous mutations in untreated animals has not been conducted. Considering these factors, and the high background incidence of mutation frequency in this study decreasing the sensitivity for detecting an effect, no inferences can be made regarding the significance of these results.

Although micronucleus detection in bone marrow or peripheral blood is standard practice, this assay can be used for any tissue with actively dividing cells. Two studies by the same group tested intact duodenal tissues from mice exposed to Cr(VI) in drinking water for 7 or 90 days, separately counting micronuclei in crypt and villous cells. Both studies, testing dose ranges based on the NTP 2-year bioassay that did not include a group with a maximum tolerated dose, reported no increased incidence of micronuclei in crypt cells from Cr(VI)-exposed animals. The first, O'Brien et al. (2013), did not observe a single micronucleus in crypt cells at any dose. The failure of establishing a background incidence, paired with no concurrent positive controls, make these

results difficult to interpret. In their second study of crypt cells, although an extremely low background incidence was observed, two exposure groups again were observed to have zero micronuclei: the top concentration (180 mg/L Cr(VI)), and one positive control, DMH (1,2-dimethylhydrazine) (Thompson et al., 2015b). This is of some concern considering this chemical has been used as a positive control to validate the micronucleus assay in the GI tract by other groups (Coffing et al., 2011; Ohyama et al., 2002; Vanhauwaert et al., 2001; Goldberg et al., 1983).

Of these two studies, only O'Brien et al. (2013) also scored villous enterocytes for micronuclei and reported a statistically significant increase at the top dose at day 7 and the two highest exposure groups (60 and 180 mg/L Cr(VI)) at day 90. Although the incidences were pooled for all animals and the total number of cells scored was not reported, this is an intriguing finding. Micronuclei cannot be formed in cells that are not actively dividing. Although intestinal villous cells have a rapid turnover rate of 3–5 days, it is the crypt cells that are the rapidly dividing progenitor cells; these cells proliferate and differentiate, migrating up the villi to form the cells lining the intestinal villi (Gelberg, 2018). The nonproliferative, fully differentiated villous enterocytes are continually sloughed into the lumen as they are replaced by new cells (Potten et al., 2009). Therefore, to discover micronuclei in the villous cells, and not in crypt cells (assuming that the study design was sufficient to detect mutational changes in this region), either demonstrates that genetic damage occurring in the crypt cells suddenly ceased or was repaired in the 24 hours between the end of the exposure and sacrifice, pushing the last micronucleated cells into the villus, or, that in response to Cr(VI), the villous enterocytes absorbing Cr(VI) began dedifferentiating and migrating back toward the crypt cells, leaving them vulnerable to the genotoxic effects of Cr(VI) (consistent with the "top-down" theory for colorectal cancer, (Hanahan, 2022; Schwitalla et al., 2013; Shih et al., 2001)). Either instance indicates a potential for Cr(VI) to induce genetic damage in intestinal villi; however, better designed experiments would be needed to draw any interpretations with confidence.

In vitro studies of GI tissues comparing genotoxicity across species have shown that cellular responses are similar in gastric mucosal cells between humans and rodents (Pool-Zobel et al., 1994). However, other genotoxicity endpoints from in vivo oral exposure studies specific to GI tissues were negative, including γH2AX, a marker of DNA double-strand breaks (Thompson et al., 2015b; Thompson et al., 2015a), and DNA-protein crosslinks were not increased in the forestomach, glandular stomach, and duodenum (De Flora et al., 2008). In addition, several in vivo studies found no increase in 8-OHdG adducts in target tissues across species (Thompson et al., 2012b; Thompson et al., 2011; De Flora et al., 2008), suggesting that oxidative DNA damage may not be a primary source of permanent DNA alteration.

Two positive but low confidence in vivo mutation studies were not conducted in portal-of-entry or tumor target tissues but were designed to detect mutations induced in germ cells and the developing fetus. Although the focus of this analysis is to inform an MOA for cancer, an agent that

causes mutation in germ cells is of added concern due to the potential for generating heritable mutations that can be passed to offspring if the agent is anticipated to reach the germinal tissues (U.S. EPA, 1986b). Marat et al. (2018) reported increased dominant lethal mutation frequency, indicative of increased chromosomal aberrations and/or gene mutations arising in the exposed F0 male. The second study found a significant dose-dependent increase in mutations in mice after gestational drinking water exposures despite elevated background frequency (Kirpnick-Sobol et al., 2006), although there are indications this study may have been biased away from the null. Although it cannot be determined from these two *low* confidence studies that ingested Cr(VI) reaches these tissues in sufficient concentrations to conclude there is a potential mutagenic hazard to germ cells and the developing fetus, further research is needed.

Although the current evidence base has not consistently identified signature mutations associated with Cr(VI) exposure, there may be some indications from in vitro studies that Cr(VI) induces mutations in vivo primarily through larger deletions or structural changes, versus smaller point mutations or frameshifts that would be detected by the transgenic rodent assay. Additional investigation of preserved tissues from animal bioassays could allow the analysis of higher numbers of cells to increase the sensitivity of micronucleus detection. Future testing for mutation induction in the GI tract could increase sensitivity by harvesting dissociated mucosal epithelial cells to increase the number of cells for analysis (Okada et al., 2019; Coffing et al., 2011), and flow cytometric scoring of micronucleated cells can dramatically increase the sensitivity of this assay (Dertinger et al., 2011). Updated technologies in DNA sequencing and the identification of mutational signatures are also capable of resolving these evidence gaps (e.g., Riva et al. (2020); Valentine et al. (2020)).

In conclusion, there is consistent and coherent evidence that a mutagenic MOA for Cr(VI)-induced carcinogenesis is biologically plausible and relevant to humans. The implications of this evidence in the context of human pharmacokinetics and the full complement of carcinogenic pathways, including interpretations regarding tissue type-specific (e.g., in the lung; in the GI tract) induction, that can be initiated by Cr(VI) exposure will be discussed in the next section.

3.2.3.4. *Mode-of-action integration of evidence for carcinogenesis*

Figure 3-16 summarizes the key events (organized by levels of biological complexity) and mechanistic pathways that have been identified to be involved in the carcinogenic process induced by Cr(VI). Evidence supporting each key event (boxes) and key event relationship (arrows) is presented in more detail in Table 3-20. The corresponding key characteristic of carcinogens (Appendix C.3.2, (Smith et al., 2016)) is identified with each key event where applicable, as well as whether the key event is recognized to be a hallmark or enabling characteristic of cancer (Hanahan, 2022; Hanahan and Weinberg, 2011). The visualization of key events in this figure resembles the layout commonly used in adverse outcome pathway (AOP) networks, but this diagram is chemical-specific. Although some events clearly precede others, due to the complexity of the key event

pathways the key events themselves have not been numbered to avoid the suggestion of an overarching temporal order.

1

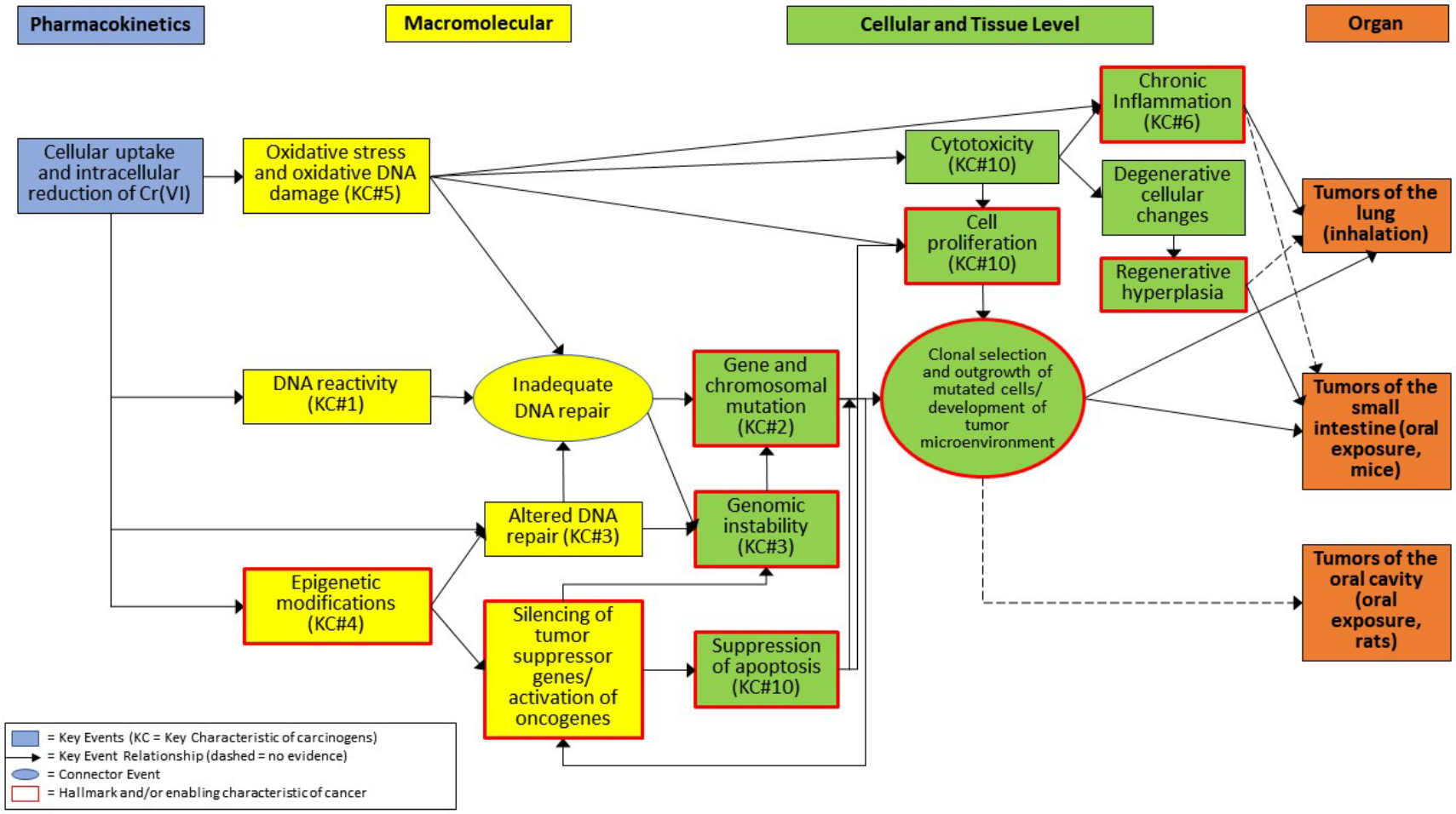

Figure 3-16. Key events and mechanistic pathways induced by Cr(VI) exposure that can lead to cancer.

Table 3-20. Evidence for key events and key event relationships involved in Cr(VI)-induced carcinogenesis

Key event	Key event relationship and evidence	References for Cr(VI)-specific evidence[a]
Pharmacokinetic-dependent molecular initiating event		
Distribution, cellular uptake and intracellular reduction of Cr(VI)	Once Cr(VI) reaches the target tissue(s) in sufficient amounts, the Cr(VI) oxyanion is taken up by cells via nonspecific anion transporters where it is reduced via intracellular reductants to Cr(V), Cr(IV), and the kinetically stable Cr(III). The predominant intracellular reduction pathways and intermediates depend on available ascorbate, glutathione, and cysteine.	Reviewed in Section 3.1.1, Zhitkovich (2011), Nickens et al. (2010) (see below summary of key events)
Macromolecular		
DNA reactivity, adduct and crosslink formation, and DNA double-strand breaks	Cr(VI) is not DNA reactive, but Cr(III), the final reduction product, can form bulky Cr-DNA and Cr-protein adducts and crosslinks, leading to replication fork stalling and DNA double-strand breaks.	Reviewed in Zhitkovich (2005) (see below summary of key events)
Oxidation of biological macromolecules and ROS generation	Redox reactions during the intracellular reduction of Cr(VI) generates reactive intermediates Cr(V) and Cr(IV) that produce reactive oxygen species, directly damaging intracellular molecules including DNA, proteins and lipids, and inducing cell signaling pathways and transcription factors associated with inflammation, cytotoxicity, apoptosis and necrosis, including TNF-α, NF-κB, and NRF2. Cr(VI) is a strong oxidizing agent and can abstract electrons from a number of intracellular ligands, forming oxyradical species and leading to oxidative stress and cytotoxicity.	Reviewed in Levina and Lay (2005), Zhitkovich (2011) (see below summary of key events)
Oxidative DNA damage	Reactive oxygen species generated by intracellular reduction of Cr(VI) can cause DNA strand breaks, both directly through free radical damage and base modifications (e.g., 8-OHdG adducts), and indirectly via ROS generation, lipid and protein peroxidation, and depletion of intracellular antioxidants and DNA repair capacity. DNA damage correlates with ROS levels and treatment with antioxidants reduces DNA damage.	Reviewed in Shi et al. (2004)

Key event	Key event relationship and evidence	References for Cr(VI)-specific evidence[a]
Epigenetic modifications	Cr(VI) exposure induces extensive promoter-specific methylation, global hypomethylation, post-translational histone modifications, and microRNA dysregulation, affecting the expression of an extensive number of genes shown to be altered by Cr(VI) exposure; this pattern of hypermethylation of CpG islands, downregulating tumor-suppressor genes, and concomitant hypomethylation of global (non-CpG) regions, upregulating tumor promoter genes, contributes to genomic instability, and has been observed in many idiopathic cancers including adenocarcinomas of the GI tract.	Reviewed in Chen et al. (2019)
Altered DNA repair	Cr(VI) exposure alters DNA repair processes by the suppression of DNA repair genes via epigenetic silencing of mismatch repair (MMR) genes. Epigenetic silencing of DNA repair genes leads to suppression of proficient DNA repair pathways, including mismatch repair (MMR), leading to microsatellite instability, and homologous recombination repair (HR), leading to an increased frequency of replication fork stalling and DNA double-strand breaks. Increased global hypomethylation and increased promoter-specific hypermethylation of CpG islands in DNA repair genes have been observed in the lung tumors of chromate-exposed workers, contributing to mutagenesis and genomic instability, a hallmark of cancer.	Reviewed in Chen et al. (2019); see also Guo et al. (2019); Wang and Yang (2019); Hu et al. (2018); Li et al. (2016); Wang et al. (2012b)
Inadequate DNA repair (connector event)	If the DNA damage produced by Cr(VI) reduction and the formation of DNA adducts and ROS damage cannot be adequately repaired (or removed by programmed cell death), this can lead to gene mutations, aneuploidy, and genomic instability. In humans, decreased DNA repair synthesis has been observed in lymphocytes among individuals exposed to chromium occupationally. The suppression of DNA damage response and repair genes increases the probability that Cr(VI)-induced genetic damage will lead to mutations.	Rudnykh and Zasukhina (1985)
Silencing of tumor suppressor genes and activation of oncogenic pathways	A number of tumor suppressor genes have been shown to be downregulated by Cr(VI) exposure, with some known to be due to epigenetic silencing, including APC, $P16^{ink4a}$, CFTR, and possibly p53, though there is conflicting evidence for p53 involvement. Activation of the c-Myc and Wnt/β-catenin oncogenic pathways has also been implicated.	Ali et al. (2011), Hu et al. (2016), Kondo et al. (2006), Tsao et al. (2011), Li et al. (2017), Lu et al. (2018), Park et al. (2017), Mezencev and Auerbach (2021)

Key event	Key event relationship and evidence	References for Cr(VI)-specific evidence[a]
Cellular and Tissue Level		
Genomic instability	Genomic and chromosomal instability induced by Cr(VI) contributes to tumorigenesis and manifests primarily as microsatellite instability, caused by the epigenetic suppression of mismatch repair genes, and aneuploidy.	Reviewed in Wise and Wise (2010); also Ali et al. (2011), Hirose et al. (2002), Peterson-Roth et al. (2005), Takahashi et al. (2005)
Gene and chromosomal mutation	Bulky Cr-DNA lesions lead to replication fork stalling and DNA double-strand breaks, which can become fixed mutations if not efficiently repaired or targeted for cell death by apoptosis. Some of these mutation may confer a growth advantage, leading to a clonal outgrowth of the mutated cells and tumorigenesis, a process that is more likely to occur in rapidly proliferating cells.	See mutagenic MOA evidence synthesis, Section 3.2.3.3
Suppression of apoptosis	Unlike the cytotoxicity-related apoptosis induced by the direct cellular injury caused by initial Cr(VI) exposures, the downstream suppression of programmed cell death via apoptosis contributes to the fixation of mutations and unchecked cell proliferation, leading to tumorigenesis. Cr(VI) was shown to initiate signaling pathways that promote cell proliferation and inhibit apoptosis in GI target tissues in rats exposed via drinking water for 60 days.	Tsao et al. (2011)
Cytotoxicity	The oxidative damage induced by Cr(VI) can lead to frank cytotoxicity, which has been observed as increased levels of apoptosis in the lung and small intestine in animals following inhalation and drinking water exposures, respectively. This cytotoxicity contributes to degenerative changes and regenerative hyperplasia. Cytotoxicity has not been detected in the rat oral cavity.	Reviewed in Levina and Lay (2005), Shi et al. (2004)
Cell proliferation	Cr(VI) exposure to the lung and GI tract has been shown to induce cell proliferation, both by inducing proliferative signaling pathways and by evading apoptotic signals that regulate uncontrolled cell growth in normal cells, contributing to hyperplasia and tumorigenesis. Increased cell proliferation can lead to increased genomic instability and the potential for the clonal selection of mutations that confer tumorigenic hallmarks. Cell proliferation has not been detected in the rat oral cavity.	Kopec et al. (2012a), Rager et al. (2017), Tsao et al. (2011), Katabami et al. (2000)
Degenerative cellular changes	Biochemical and histopathological evidence of cellular injury has been observed in the rat lung following inhalation exposures and in the mouse and rat small intestine following drinking water exposures, indicative of degenerative changes that can initiate compensatory cell proliferation. No observations of degenerative cellular changes have been observed in the rat oral cavity.	Glaser et al. (1990), NTP (2007), NTP (2008), Thompson et al. (2011), Thompson et al. (2012b)

Key event	Key event relationship and evidence	References for Cr(VI)-specific evidence[a]
Regenerative hyperplasia	Hyperplasia consistent with regeneration following cell injury has been reported following oral exposures in the small intestine of mice and rats and following inhalation exposures in the lung in rats. Hyperplasia has not been observed in the rat oral cavity following Cr(VI) exposures.	NTP (2008), NTP (2007), Glaser et al. (1990), Thompson et al. (2011), Thompson et al. (2015b), Thompson et al. (2012b)
Inflammation	Chronic inflammation is an enabling characteristic of cancer. Evidence consistent with inflammatory lung responses has been observed following Cr(VI) inhalation. However, no evidence consistent with chronic inflammation has been reported following oral exposures in animals or humans. Some suggestive evidence from oxidative stress, cytokine fluctuations, and proinflammatory signaling pathways (e.g., NF-kB) may be indirectly indicative but this evidence in inconclusive.	Johansson et al. (1986b), Glaser et al. (1990), Glaser et al. (1985), Cohen et al. (2003), Kim et al. (2004)
Organ		
Tumor formation	• Lung (inhalation): Cr(VI) is a human lung carcinogen. • Oral cavity (ingestion): Increased incidence of squamous cell carcinomas or papillomas (mucosa or tongue) in both sexes of F344/N rats (NTP 2-year bioassay). Statistically significant at highest dose (≥6 mg/kg-d in males, ≥ 7.13 mg/kg-d in females) with dose-response trend in lower dose groups, in drinking water. See Figure 3-16 and Table 3-15. Tumors are rare (see Appendix D.5). • Small intestine (ingestion): Increased incidences of adenomas and carcinomas in both sexes of B6C3F1 mice (NTP 2-year bioassay). Statistically significant at two highest exposures (≥ 2.4 mg/kg-d in males, ≥3.2 mg/kg-d in females) with dose-response trend in lower dose groups, in drinking water. See Figure 3-16 and Table 3-15. Tumors are rare (see Appendix D.5).	NTP (2008)

[a]Complete references for the evidence provided in the table can be found in the below summaries of each key event.

Key events for Cr(VI)-induced cancer

Pharmacokinetic-dependent Molecular Initiating Event: The distribution, cellular uptake and reduction of Cr(VI)

The effects induced by Cr(VI) can only occur if Cr(VI) reaches the target tissue prior to extracellular reduction, which essentially inactivates its toxic and carcinogenic potential. Therefore, consideration of the pharmacokinetics and the competing processes of reduction and uptake of inhaled or ingested Cr(VI) are central to assessing the carcinogenic potency of Cr(VI). Chromium (VI) compounds have been traditionally considered nonreactive towards purified DNA under physiological conditions. Their ability to induce oxidative stress and DNA damage in exposed cells and tissues in vitro and in vivo (discussed in the following sections) is explained by the uptake-reduction model of Cr(VI)-mediated genotoxicity (Wetterhahn et al., 1989). Based on this model and irrespective of target cell type, Cr(VI) species taken up by cells by anion transporters undergo intracellular reduction predominantly driven by ascorbate, glutathione and cysteine to form DNA-reactive and/or oxidative damage-inducing intermediates Cr(V) and Cr(IV), and eventually the thermodynamically stable Cr(III), which accumulates in cells via its binding to DNA and other molecules (Zhitkovich, 2011, 2005). These nonspecific anion transporters, present in all cell types, rapidly take up soluble Cr(VI) due to the structural similarity of the tetrahedral configuration of the chromate ($Cr_2O_4^{2-}$) or dichromate ($Cr_2O_7^{2-}$) anions to that of phosphate (HPO_4^{2-}) and sulfate (SO_4^{2-}) anions (Wetterhahn et al., 1989).

Reduction of Cr(VI) is a kinetically controlled process, and the role of specific reductants reflects their reaction rates with Cr(VI) compounds and intracellular concentrations. The highest rate of Cr(VI) reduction was found for ascorbate, followed by cysteine and glutathione with respective rate ratios of 61:13:1 (Quievryn et al., 2003). Since typical intracellular concentrations of ascorbate (1-2 mM) and glutathione (1-10 mM) are comparable and considerably higher than that of cysteine (0.03-0.2 mM) (Tian et al., 2014), the principal intracellular reducer of Cr(VI) is ascorbate, accounting for 80-90% of its metabolism (Zhitkovich, 2011, 2005). Ascorbate and glutathione also display a synergistic effect on the reduction of Cr(VI), and the rate of this reduction by a mixture of ascorbate and glutathione under physiologically relevant conditions was found higher than a sum of reduction rates of each of these reductants (Suzuki, 1990).

It should be noted that studies performed in cell-free or cell-based systems that do not fully reflect physiological conditions and concentrations of intracellular reducers may not truly reflect cellular and molecular processes that occur in human tissues under environmental exposures to Cr(VI). This limitation affects mechanistic cell-free studies that use certain non-physiological buffers and cell-based studies that employed ascorbate-depleted cells grown in standard growth media (Quievryn et al., 2002). Since ascorbate represents a major intracellular reductant of Cr(VI) (Suzuki and Fukuda, 1990), restoration of ascorbate in cell-based systems is necessary for a correct assessment of the fate of Cr(VI) and DNA damage following its intracellular uptake.

Reduction of Cr(VI) by ascorbate generates variable amounts of Cr(V), Cr(IV), and carbon-based radicals (Stearns and Wetterhahn, 1994). At physiologically relevant molar ratios of ascorbate to Cr(VI) exceeding 2:1, the only detectable intermediate reduction product is reportedly Cr(IV). The presence of Cr(V) is detectable only at non-physiological ratios of equimolar or lower ratio of ascorbate to Cr(VI), or in ascorbate-depleted cells (Zhitkovich, 2011; Stearns and Wetterhahn, 1994). Reduction of Cr(VI) by ascorbate under physiologically relevant conditions is a low oxidant-generating process that differs remarkably from reduction of Cr(VI) by glutathione, which generates substantially more reactive oxygen species (Wong et al., 2012). However, in spite of reduced DNA oxidative damage in cells with restored ascorbate, these cells can still experience a large increase in genotoxicity, as displayed by an increased frequency of DNA double-strand breaks in one study by Wong et al. (2012).

The reduced form of glutathione (GSH) is a major intracellular reducer of Cr(VI) in cells cultured without restoration of ascorbate (Figure 3-7 in Section 3.1.1). This reduction can be a one- or two-electron process (Zhitkovich, 2011), but more typically it proceeds as a one-electron process sequentially producing Cr(V), Cr(IV) and Cr(III) (Marin et al., 2018). Reduction by cysteine in the presence of variable amounts of glutathione is also a one- or two-electron process, with the one-electron process dominating in the physiological range of concentrations (Quievryn et al., 2001).

As described in Section 3.1.1.2, inhaled Cr(VI) that deposits in the upper and lower respiratory tract will come in direct contact with epithelial cells. Reduction of Cr(VI) by epithelial lining fluid is less effective than gastric fluid, and both high and low-soluble compounds can pose a hazard to respiratory tract epithelial cells. Although highly soluble Cr(VI) compounds may clear the lungs faster than low-soluble forms, they have the potential to be more readily taken up by cells. Low-soluble forms are absorbed more slowly and may be cleared in the mucus but may expose the epithelial cells for a longer period of time. In addition, high localized accumulation of Cr(VI)-containing particulates may occur in susceptible lung regions such as airway bifurcation sites (Balashazy et al., 2003; Schlesinger and Lippmann, 1978). This is supported by studies showing high chromium deposition at these sites in the lungs of chromate workers, and a correlation between lung chromium burden and lung cancer (Kondo et al., 2003; Ishikawa et al., 1994a, b). There is an extensive mechanistic database demonstrating the toxicity and mutagenicity of Cr(VI) in humans via the inhalation route of exposure (see Section 3.2.3.3 and Appendix C.3.2.2). Therefore, it will be assumed that inhaled Cr(VI) at any concentration is capable of exposing the epithelial cells in the respiratory tract, and that compared to GI epithelial cells after Cr(VI) ingestion (discussed below), the respiratory epithelial cells have an increased potential for Cr(VI) uptake and Cr(VI)-mediated cytotoxicity and the induction of mutations in these cells.

Following ingestion, the evidence shows that approximately 10% of the Cr(VI) dose is absorbed in the GI tract of rodents (Fébel et al., 2001; Thomann et al., 1994). In humans, it is estimated that <10% is absorbed in the GI tract (depending on the dose and stomach pH), and this number may be 10% or higher in susceptible populations (see Section 3.3.1 and Appendix C.1.5).

Therefore, it is likely that a portion of ingested Cr(VI) interacts with the epithelial cells of the GI tract in all species. Effects observed by NTP (2008) in mice indicate that unreduced Cr(VI) may traverse the entire small intestine. The highest incidences of tumors and potentially preneoplastic lesions were observed in the duodenum, the region most proximal to the stomach. This region has a higher surface area per unit length of intestine (Casteleyn et al., 2010), increasing the absorptive capacity in this tissue. The combination of high Cr(VI) concentration at the epithelial surface and high absorptive surface capacity are the likely main contributors to the lesions observed in mice by NTP (2008).

In contrast to the duodenum, the absorption surface area of the stomach is low (Casteleyn et al., 2010), which may account for the lack of stomach tumors in the NTP (2008) bioassay. The jejunum and ileum have lower absorption surface areas than the duodenum (but still higher than the stomach), and these segments exhibited lower incidences of tumors in mice than the duodenum. Lower tumor incidence also may have been a result of Cr(VI) reduction and dilution by intestinal secretions and lumen contents. Data by Kirman et al. (2012) shows chromium concentrations decreasing in the distal direction in the small intestine of mice exposed to Cr(VI) in drinking water for 90 days. While the absorption surface area of the oral cavity is also low, as the first tissue of contact, it is being exposed to the highest concentration of Cr(VI). This may make oral tissues more prone to neoplastic effects in rats. However, pharmacokinetics cannot explain why rats and mice differ with respect to oral and small intestinal tumors, since these differences may be due to a variety of other factors (Ibrahim et al., 2021; Chandra et al., 2010). Figure 3-17 illustrates the ordering of tissues within the GI tract and is annotated with the types of tumors observed by NTP (2008) in both mice and rats.

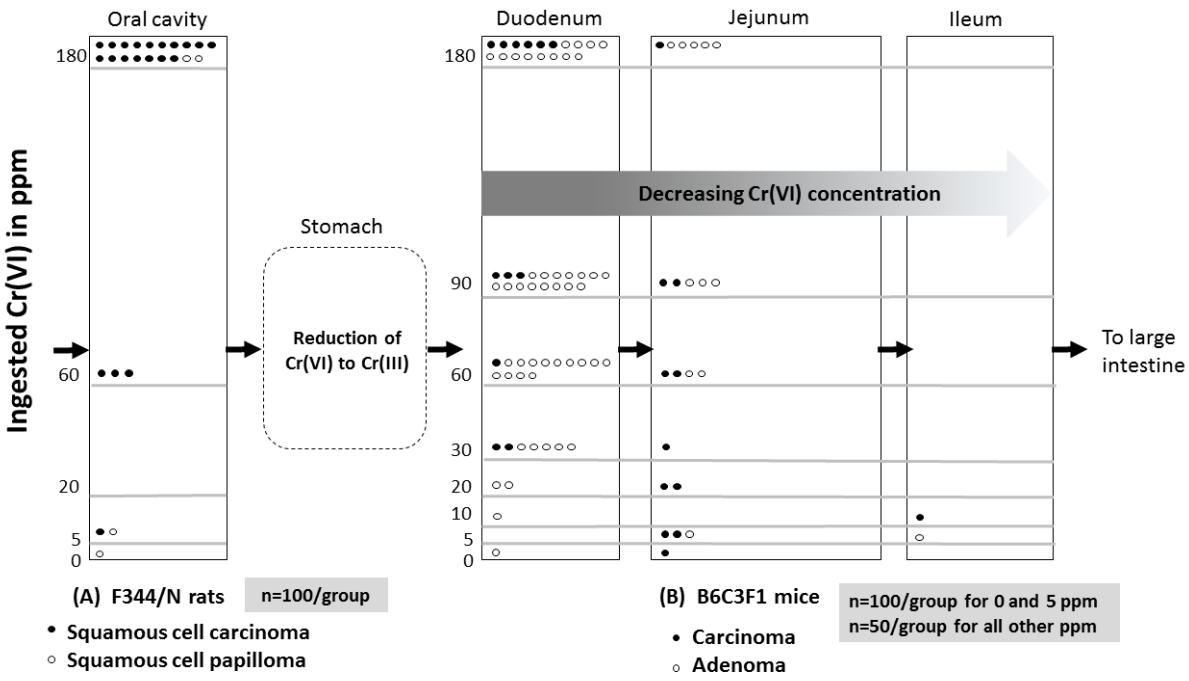

Figure 3-17. Reported tumors of the digestive tract tissues for all rodents exposed to Cr(VI). Points indicate primary adenomas and carcinomas for the mouse small intestine, and primary squamous cell carcinomas and squamous cell papillomas for the rat oral cavity (oral mucosa and tongue). Multiple tumors per animal per tissue are included, but tumors which were known to have metastasized from other sites were not included.

In the small intestine, the localization of total chromium in different intestinal compartments provides some mechanistic information on the ability of Cr(VI) to reach the crypts (where stem cells reside), which could give rise to cytotoxicity as well as fixed mutations in these highly proliferative cells. Thompson et al. (2015b; 2015a) used X-ray fluorescence microspectroscopy to examine the concentrations of total chromium in the cells residing within mouse villi and crypts after 1 and 13 weeks of exposure. All analysis was performed in the middle section of the duodenum, which may be a significant source of bias because (1) ingested Cr(VI) tissue concentrations are expected to be highest in the section of the duodenum closest (proximal) to the stomach because reduction/dilution will occur as Cr(VI) traverses the intestine, and (2) the human duodenum is much shorter than that of the rodent duodenum (Casteleyn et al., 2010), and therefore the middle section of the rodent duodenum may not be as relevant to humans. After 13 weeks of exposure, Thompson et al. (2015a) detected a weak Cr signal (0.4 µg/g) in the 24 small intestine crypts that were examined, with a 35-fold higher (14 µg/g) mean concentration in the villi. A separate 7-day study reported the absence of Cr in the crypt compartment without quantitative results; however, these observations may be biased toward the null due to the rapid movement of cells from the crypt compartment and the 24-hour recovery time before imaging was performed (Thompson et al., 2015b). In a subsequent gene expression study that separately analyzed

microdissected crypts and villi in preserved mouse small intestinal tissues from Thompson et al. (2011), a robust response in gene expression changes was detected in crypts at ≥4.6 mg Cr(VI)/kg-d and in villi at all doses (≥0.024 mg Cr(VI)/kg-d) after 7 and 90 day exposures, demonstrating that Cr(VI) does reach the crypts at these concentrations in drinking water (Chappell et al., In Press).

In light of the pharmacokinetic evidence, this assessment assumes that ingested Cr(VI) escaping stomach reduction is capable of coming into contact with cells of the epithelium of the lower GI tract (small and large intestine), although the Cr(VI) concentration exposing the cells will be lower than the ingested concentration. Furthermore, this assessment assumes that ingested Cr(VI) at any concentration is capable of coming into direct contact with the epithelial cells of the upper GI tract (oral cavity, esophagus, and stomach) prior to stomach reduction. The Cr(VI) concentration exposing the cells of the oral cavity is likely very close to the ingested concentration.

DNA reactivity (KC#1)

Cr(VI) is not known to be DNA reactive. In contrast, the intermediate Cr(IV) and Cr(V) and terminal Cr(III) species, generated during intracellular reduction of Cr(VI), can induce DNA damage through their direct interactions with DNA, or indirectly via oxidative damage (Arakawa et al., 2012). Reduction of Cr(VI) in cell-free, cell-based and in vivo systems generates variable amounts of the intermediate chromium species depending on the nature and concentration of the reducers, concentrations of Cr-species, and other conditions (Borges et al., 1991). It has been suggested that the abundance of specific intermediate species could be a major factor in determining the DNA damaging activity of Cr(VI) (Sugden and Stearns, 2000). The kinetics of intracellular reduction are reviewed in Section 3.1.1.3, and the specific experimental support for the in vivo generation of the intermediate and terminal Cr species, as well as their direct and indirect genotoxicity potential, is described in Appendix C.3.2.

DNA reactivity of Cr species

Formation of Cr(V) and free radicals generated by these species has been considered to play an important role in Cr(VI)-induced DNA damage. Findings reported by Shi and Dalal (1994) were interpreted as evidence for the ability of Cr(V) to interact with the N7 atom of purines to form Cr(V)-dG and Cr(V)-dA DNA adducts and cause oxidative damage at dG sites and formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) residues, presumably caused by hydroxyl radicals generated through a Fenton-like reaction (i.e., $Cr(V)+H_2O_2 \rightarrow Cr(VI) + \cdot OH + OH^-$). In addition to the oxidative damage through reactive oxygen species, Cr(V) has been shown to induce direct oxidative DNA damage through abstraction of H atoms at the deoxyribose sugar moiety, which results in generation of abasic sites (Sugden and Wetterhahn, 1997).

Cr(IV) is the major transient form of intracellular reduction of Cr(VI) in cells with physiological levels of ascorbate. In the presence of hydrogen peroxide, Cr(IV) is a more potent Fenton-like reagent than Cr(V) and generates hydroxyl radicals, which has been shown to cause DNA strand breaks (Luo et al., 1996) and oxidative damage at dG positions, which are preventable

by hydroxyl radical scavengers (Shi et al., 1999). In addition, this process generates Cr(V), which can cause DNA damage through direct and indirect mechanisms.

Cr(III) is a thermodynamically stable species produced by the reduction of Cr(VI) through the intermediary species Cr(V) and Cr(IV), which transiently exist in variable amounts during the intracellular reduction of Cr(VI). The interaction of Cr(III) with DNA is responsible for the formation of DNA lesions, the most common of which are the binary Cr(III)-DNA adducts (Floro and Wetterhahn, 1984). Two different forms of Cr(III)-DNA adducts were suggested by a study that examined DNAzyme Ce13d reacted with $CrCl_3$. The results showed that Cr(III) first binds to the DNA phosphate backbone through weak electrostatic interactions, then slowly coordinates with all four nucleobases, forming highly stable DNA interstrand crosslinks (Zhou et al., 2016). A more recent study concluded instead that Cr(III) is coordinated with N7 of dG as a $[Cr(H_2O)_5]^+$ complex located within the major groove of the DNA double helix structure without the direct participation of neighboring bases of phosphate groups (Brown et al., 2020), but also supported the formation of interstrand crosslinks. It is likely that the existing evidence of the reactions of Cr(III) complexes with DNA do not provide a full model of all possible Cr-DNA interactions that occur during Cr(VI) reductions with variable amounts of intracellular reducers.

Binary Cr(III)-DNA adducts can further conjugate proteins and form DNA-protein cross-links (DPCs). The DPCs represent ternary protein-Cr(III)-DNA adducts generated by a rate-limiting reaction of binary Cr(III)-DNA adducts with proteins. Formation of DPCs in cultured cells exposed to Cr(VI) is decreased by depletion of glutathione and facilitated by restoration of physiological levels of ascorbate (Macfie et al., 2010). Overall, the biological significance of the DPCs is still incompletely understood. In addition to inducing possible genotoxic responses, some studies demonstrated their ability to inhibit specific gene expression (Macfie et al., 2010).

Other ternary adducts identified in the cells exposed to Cr(VI) are ascorbate-Cr(III)-DNA, glutathione-Cr(III)-DNA, cysteine-Cr(III)-DNA, and histidine-Cr(III)-DNA. Ascorbate-Cr(III)-DNA adducts were detected in Cr(VI)-treated human A549 lung cancer cells with restored ascorbate levels, and these crosslinks accounted for approximately 6% of the total DNA-bound chromium (Quievryn et al., 2002). In addition, binding of Cr(III) and the formation of Cr(III)-DNA adducts induces structural distortions of DNA (Zhitkovich et al., 2001).

Biological effects of Cr-DNA interactions

Binary Cr(III)-DNA adducts formed by the reaction of Cr(III) aqua complexes and DNA are reportedly weakly mutagenic lesions and their mutagenic potential is considerably lower in comparison with any ternary ligand-Cr-DNA adduct (Quievryn et al., 2003). Indeed, ascorbate-Cr(III)-DNA and cysteine-Cr(III)-DNA adducts were found to be 31-fold and 5.3-fold more mutagenic than the binary Cr(III)-DNA adducts, respectively (Holmes et al., 2008; Zhitkovich et al., 2001). Consequently, ascorbate appears to be the most important intracellular reducer of Cr(VI) that forms highly mutagenic DNA adducts. The ternary adducts glutathione-Cr(III)-DNA and histidine-Cr(III)-DNA were also found mutagenic, and their mutagenicity exceeded that of cysteine-

Cr(III)-DNA (Voitkun et al., 1998). Ternary adducts are also more genotoxic than binary Cr(III)-DNA adducts, demonstrated through more prominent DNA replication fork stalling by ternary adducts in comparison to binary adducts (e.g., (Quievryn et al., 2003; Snow and Xu, 1991).

Under lower, non-physiological levels of ascorbate, reduction of Cr(VI) by glutathione in vitro produced mutagenic glutathione-Cr(III)-DNA adducts (Guttmann et al., 2008). This finding implies weak mutagenicity of lesions produced at physiological concentrations of GSH in ascorbate-depleted cells and suggests that studies employing standard cell cultures with low intracellular ascorbate could have underestimated mutagenicity of Cr(VI). Taken together, studies performed under non-physiological low ascorbate levels favored production of Cr(V) and a lower amount of highly mutagenic ternary species, which did not truly reflect the genotoxic and mutagenic effects of Cr(VI) (Quievryn et al., 2006).

Cells with restored ascorbate levels display considerably different cell signaling responses to Cr(VI) than ascorbate-depleted cells. As previously shown, reduction of Cr(VI) by glutathione in vitro and in cells with depleted ascorbate leads to an appreciable formation of Cr(V), which can act as an oxidant (Quievryn et al., 2003), while reduction of Cr(VI) by ascorbate is a low oxidant generating process (Wong et al., 2012). Treatment with Cr(VI) also induces double-strand breaks in cells with restored ascorbate; however, these are formed selectively in euchromatin and their signaling is dependent on ATR rather than on ATM kinase (Deloughery et al., 2015).

The specific role of Cr-species and Cr-induced DNA lesions in the toxicity and carcinogenicity of Cr(VI) has not yet been conclusively established. Depending on experimental conditions, reduction of Cr(VI) has been found to produce binary Cr-DNA and ternary ligand-Cr-DNA adducts, interstrand crosslinks, DNA-protein crosslinks, oxidative damage to bases and deoxyribose, DNA strand breaks, and DNA abasic sites, which have been associated, to various extents, with cell cycle arrest, DNA repair, cell death and mutagenesis (Sugden et al., 2001; Arakawa et al., 2000; Casadevall et al., 1999; Stearns and Wetterhahn, 1997; Zhitkovich et al., 1996b; Bridgewater et al., 1994).

Oxidative stress and oxidative DNA damage (KC#5)

Oxidative stress induced by Cr(VI) exposure appears to lead to several toxicity pathways causing cytotoxicity, inflammation (in the lung), cell proliferation, and DNA damage. Redox reactions during the intracellular reduction of Cr(VI) generate reactive intermediates Cr(V) and Cr(IV) that produce reactive oxygen species, which can cause cytotoxicity and directly damage intracellular molecules including DNA, proteins and lipids, and in the process, induce cell signaling pathways associated with inflammation and cell proliferation (reviewed in Levina and Lay (2005)). Radical species formed when Cr(VI) oxidizes intracellular macromolecules can also induce oxidative damage (reviewed in Zhitkovich (2011)). Reactive oxygen species generated by intracellular reduction of Cr(VI) can cause free radical damage to DNA via base modifications (e.g., 8-OHdG adducts), lipid and protein peroxidation, and depletion of intracellular antioxidants (reviewed in Shi et al. (2004)). Because these effects have been well-documented in review articles,

this section will focus on evidence of oxidative stress in occupationally exposed humans and in animals exposed to Cr(VI) via oral or inhalation, or in vitro studies using human cells derived from lung or GI tissues. Oxidative stress induced by Cr(VI) exposure has been characterized in other health effects sections of this assessment, including oxidative damage contributing to Cr(VI)-induced toxicity of the lung (Section 3.2.1), GI tract (Section 3.2.2), liver (Section 3.2.4), male and female reproductive organs (Sections 3.2.7 and 3.2.8, respectively), and fetal development (Section 3.2.9). Therefore, the evidence from the lung and GI tract in animals will be briefly summarized again here, along with systemic evidence of oxidative stress following inhalation or oral exposures.

As summarized in Section 3.2.1, many observational studies reported statistically significantly increased incidences of systemic disruption in cellular redox status that correlated with exposure to Cr(VI) in urine and blood of industrial workers and rodents exposed to Cr(VI); these are also summarized in Appendix C.3.2.5. In tumor target tissues, one study relevant to lung tissues did not detect increased 8-OHdG adducts in the sputum of lead chromate pigment factory workers (Kim et al., 1999). No studies examining oxidative stress in GI tissues were identified in exposed humans.

A small number of animal studies were identified that evaluated oxidative stress in tumor target tissues. Oxidative DNA damage in the rat lung, evidenced by increased formation of 8-OHdG adducts, was reported following inhalation or intratracheal instillation exposures in rats (Zhao et al., 2014; Maeng et al., 2003; Izzotti et al., 1998). Three in vivo studies were identified that reported biomarkers of oxidative stress in GI tissues after oral exposure (Thompson et al., 2012b; Thompson et al., 2011; De Flora et al., 2008). None of these studies observed an increase in 8-OHdG adducts in the mouse or rat small intestine or oral cavity following Cr(VI) drinking water exposures. However, an increased proportion of oxidized glutathione (GSSG) relative to reduced glutathione (GSH), indicative of oxidative stress, was observed in the mouse small intestine after 7 and 90 days of exposure, with a correlated change in the GSH/GSSG ratio in plasma after 90 days at doses ≥59 mg/L Cr(VI) (Thompson et al., 2011). A decreased GSH/GSSG ratio was also observed in the mouse oral mucosa after 7 days, but this resolved after 90 days despite a significantly higher total chromium concentration in these tissues compared with the control (Thompson et al., 2011). Changes in GSH/GSSG ratios were generally not observed in the oral cavity of rats after 7 days of Cr(VI) exposure (the ratio was decreased at 0.1 mg/L Cr(VI) in the oral mucosa) but were significant and dose-dependent in the oral mucosa and jejunum (and not the duodenum) at ≥20 mg Cr(VI)/L for 90 days (Thompson et al., 2012b), with a significantly decreased ratio in plasma at ≥170 mg/L. While GSH/GSSG ratio measurement is a generally accepted indicator of oxidative stress, ascorbate is the preferred in vivo reductant, accounting for 90% of Cr(VI) oxidative metabolism. Therefore, though the primary oxidative pathway is not captured in these experiments, the level of involvement of GSH implies extensive oxidative stress was occurring in these tissues. Other indicators of protein or lipid oxidation were not elevated in the duodenum of mice after 90 days (Thompson et al., 2011) or in the rat in the oral mucosa or duodenum (Thompson et al.,

2012b). The reason for the lack of oxidative DNA lesions associated with the oxidative stress in these studies is not known. The significance of the oxidative stress detected in tissues that do not develop tumors, or the potential physiological reasons for the inconsistencies between species, is also not clear.

A large body of evidence from cells exposed in vitro exists to support and investigate the oxidative damage induced by Cr(VI) (Appendix Table C-57). These studies include tests in model systems where ROS levels, lipid and protein oxidation, and decreased levels of antioxidant enzymes all correlate with DNA damage. This DNA damage is increased in test systems deficient in processes involved in repairing free radical damage, and is decreased in many test systems with antioxidant pre-treatment. The evidence base includes studies performed with human lung or colon and gastric cancer cell lines to study oxidatively induced DNA damage and cytotoxicity. These in vitro studies have been summarized in "Mechanistic Evidence" in Sections 3.2.1 (Respiratory Tract Effects Other Than Cancer) and 3.2.2 (Gastrointestinal Tract Effects Other Than Cancer).

In addition to oxidative stress initiating cytotoxicity and DNA damage following Cr(VI) exposure, there is evidence that oxidative stress can result in pro-inflammatory signaling pathways that contribute to cancer. The nuclear transcription factor NF-κB is activated in response to redox cell signaling and cytokines and is involved in cell survival, proliferation and inflammation (Taniguchi and Karin, 2018). NF-κB has been found to be upregulated in response to Cr(VI) exposure in numerous studies and test systems, including in the Cr(VI)-exposed rat lung (Zhao et al., 2014), in human lung cells in vitro (Wang et al., 2019; He et al., 2013; Zuo et al., 2012; Wang et al., 2004; Kim et al., 2003), and in other human cells in vitro (Tully et al., 2000; Kaltreider et al., 1999; Chen et al., 1997; Ye et al., 1995). The increases in NF-κB levels correlated with increasing ROS levels and were abrogated by antioxidant treatments (Kim et al., 2003; Chen et al., 1997). Increased NF-κB expression was shown to prevent apoptosis induced by Cr(VI) exposure in human lung cells, potentially contributing to oncogenic transformation (Wang et al., 2004). TNF-α, which activates NF-κB, is a pro-inflammatory cytokine produced by immune cells that are involved in redox signaling (Blaser et al., 2016). It has been shown to be induced systemically by Cr(VI) in rats (Mitrov et al., 2014), in LPS-stimulated mice (Jin et al., 2016), and in HaCaT immortalized human keratinocyte cells in vitro (Lee et al., 2014; Wang et al., 2010b). However, these findings were not predictive of the results in three studies of occupationally exposed humans, which did not detect increased systemic TNF-α levels in blood or serum (Qian et al., 2013; Mignini et al., 2009; Kuo and Wu, 2002).

The transcription factor NRF2 binds to and activates genes regulated by Antioxidant Response Element (ARE) in response to oxidative stress, transactivating genes for antioxidant enzymes and promoting cell survival (He et al., 2020). NRF2 has been observed to be upregulated in human liver cells (Zhong et al., 2017a) and constitutively activated in Cr(VI)-transformed human lung cells in vitro (Clementino et al., 2019). In vivo, the gene that codes for NRF2, NFE2L2, was

found to be upregulated in the duodenum of mice exposed for 91 days to Cr(VI) in drinking water (Kopec et al., 2012a).

Gene expression changes in genes involved in ROS homeostasis have also been observed in human lung, hepatic, and epithelial cells treated with Cr(VI) in vitro (e.g., NOX, SOD1, SOD2, CAT, GSR) (Zhong et al., 2017b; Zhong et al., 2017a; Zeng et al., 2013; Russo et al., 2005; Asatiani et al., 2004). In addition, Cr(VI) was found to oxidize and inhibit mitochondrial and cellular thioredoxins and peroxiredoxins involved in cell survival and redox signaling in immortalized human bronchial epithelial cells, leading to increased sensitivity to ROS damage (Myers et al. (2011; 2010; 2009; 2008)).

Overall, there is a consistent, coherent, and biologically plausible evidence base available to describe the intracellular reduction and redox imbalance, oxidative stress, and cellular oxidative damage due to free radical generation caused by Cr(VI) exposure, potentially contributing to cytotoxicity, genetic damage, and cell proliferative signaling pathways.

Epigenetic modifications (KC#4)

Epigenetic modifications are heritable changes in gene expression that occur without altering the genetic material (Sharma et al., 2010). This "nonmutational epigenetic reprogramming," which can be mediated through modifications to histones, DNA methylation, and noncoding RNAs (e.g., microRNA), is considered an enabling characteristic of cancer (Hanahan, 2022). Five studies evaluated epigenetic changes in humans in relation to chromium exposure. Kondo et al. (2006) reported increased methylation of $P16^{ink4a}$, a tumor-suppressor gene, in chromate factory workers with lung cancer who had occupational chromate exposure compared to those without chromate exposure. Similarly, they observed increased methylation of $P16^{ink4a}$ with increased duration of chromium exposure (≥15 years) among lung cancer cases (Kondo et al., 2006). Increased methylation was also observed in DNA MMR genes hMLH1 and hMSH2 when comparing lung cancer cases with and without chromate exposure (Ali et al., 2011; Takahashi et al., 2005) and in the CpG islands (promoter regions) of MMR and HR genes (i.e., MGMT, HOGG1, XRCC1, ERCC3, and RAD51) in exposed factory workers compared to controls (Hu et al., 2018). Another study identified inverse associations between blood chromium and the microRNA miR-3940-5p, which functions as an epigenetic tumor-suppressor by targeting cyclin D1 and ubiquitin specific peptidase-28 (Ren et al., 2017), as well as between miR-3940-5p and the DNA repair genes BRCC3 and XRCC2, involved in DNA damage response and homologous DNA repair (Li et al., 2014b). Ali et al. (2011) also observed increased methylation at MGMT, which encodes an enzyme that repairs DNA adducts at the O6 position of guanine, in chromate lung tumors compared to non-chromate lung tumors, as well as in APC, a tumor-suppressor gene that is suppressed via promoter hypermethylation or mutation in over 85% of colorectal cancers (Zhu et al., 2021; Juanes, 2020). Two additional studies reported decreased methylation across global DNA (Wang et al., 2012b) as well as mitochondrial genes (MT-TF and MT-RNR1) specifically (Linqing et al., 2016) in chromium-

exposed workers (chromate production workers and chrome-plating workers, respectively) compared to controls.

The findings in humans are supported by studies in vitro showing that Cr(VI) exposure induces extensive promoter-specific hypermethylation, global hypomethylation, post-translational histone modifications, and microRNA dysregulation, demonstrating that Cr(VI)-mediated epigenetic alterations may play a role in affecting the expression of an extensive number of genes shown to be altered by Cr(VI) exposure (reviewed in Chen et al. (2019)). The results from toxicogenomic studies (reviewed in Appendix C.3.4) showing multiple pathways affected by Cr(VI) with relevance to carcinogenesis are consistent with the scope of genes shown to be affected by Cr(VI)-induced epigenetic alterations. This pattern of hypermethylation of CpG islands and concomitant hypomethylation of global (non-CpG) regions has been observed in many idiopathic cancers including adenocarcinomas of the GI tract (Locke et al., 2019; CGARN, 2018a).

Altered DNA repair (KC#3)

Although there are numerous processes contributing to the repair of genetic damage when it occurs, these processes are not failsafe, and any alterations to these activities can result in an increased risk of heritable mutation (Chatterjee and Walker, 2017). As reviewed in the next section, epigenetic modifications induced by Cr(VI) exposure have been shown to silence genes involved in DNA repair, an effect that is found in a significant number of lung tumors from chromate workers compared to non-chromate lung tumors and has been found to increase with dose (Hu et al., 2018; Li et al., 2014b; Ali et al., 2011; Takahashi et al., 2005). Hirose et al. (2002) reported finding microsatellite instability (MSI) at two or more loci in 78.9% of lung cancers with chromate exposure compared to lung cancers without chromate exposure. MSI is the result of a state of genetic hypermutability that is caused by defective mismatch repair and is found in approximately 15% of colorectal cancers (Boland and Goel, 2010). Subsequent studies identified hypermethylation of the CpG island promoter regions of MMR genes hMLH1 and hMSH2 in lung tumors of workers exposed to chromate compared to lung tumors from unexposed subjects (Ali et al., 2011; Takahashi et al., 2005). In vitro, Cr(VI) exposure of human colon cells lacking MLH1 protein led to increased resistance to apoptosis, providing a selective growth advantage (Peterson-Roth et al., 2005). This epigenetic silencing of genes involved in DNA repair observed in workers exposed to Cr(VI) may contribute to mutagenesis and genomic instability, a hallmark of cancer.

Another study of workers in the chromium industry investigated the effect of prolonged exposure to Cr(VI) on the ability of the cell to correct errors during DNA replication. Evidence of decreased DNA repair synthesis was observed in isolated lymphocytes exposed to UV light to compare DNA repair synthesis between Cr(VI)-exposed workers and unexposed subjects (Rudnykh and Zasukhina, 1985). A nonmonotonic relationship with duration of exposure was also identified, though sample size was limited within each category of duration.

This slowing of DNA replication could be explained by the formation of bulky Cr-DNA adducts, which can stall replication forks, leading to increased formation of DNA double-strand

breaks. There are two main DNA double-strand break repair pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). NHEJ is the predominant repair process in the G1 phase of the cell cycle, prior to synthesis, when only one chromatid is present; it is more error-prone than HR, which occurs primarily in S/G2, using the sister chromatid as a template for repair. Cr(VI) has been shown to induce DNA double-strand breaks and Rad51 foci formation, inducing HR in vitro (Bryant et al., 2006). However, several studies have also reported a specific inhibition of genes involved in HR, including Rad51 (Hu et al., 2018; Browning et al., 2016; Li et al., 2016). Cr(VI)-induced targeting of Rad51 following prolonged in vitro exposures to Cr(VI) has also been shown to involve alterations in Rad51-mediated nucleofilament assembly, which the authors speculated was due to a Cr(VI)-mediated inhibition of Rad51 nuclear import (Browning and Wise, 2017; Browning et al., 2016). This evidence suggests that a Cr(VI)-mediated influence on Rad51 may result in modifications to HR, increasing reliance on NHEJ and potentially leading to unrepaired DNA double-strand breaks and increased aneuploidy and genomic instability.

Silencing of tumor suppressor genes and activation of oncogenic pathways

The ability to evade growth inhibition by suppressing genes that limit cell proliferation is a hallmark of cancer (Hanahan and Weinberg, 2011). The decreased expression of a number of tumor suppressor genes has been observed following Cr(VI) exposure. For some of these genes, the mechanism of decreased expression involves epigenetic silencing, and it has been observed that GI tumors have significantly higher frequencies of DNA hypermethylation at CpG islands than non-GI tumors (CGARN, 2018a). Cr(VI) was found to induce methylation at CpG sites in the promoter region of the $P16^{ink4a}$ tumor-suppressor gene; inactivation of this gene is commonly found in lung cancers and was observed in lung tumors of workers exposed to chromate, which increased with duration of exposure (Hu et al., 2016; Ali et al., 2011; Kondo et al., 2006). Methylation of the APC (adenomatous polyposis carcinoma) gene, a tumor-suppressor gene that maintains genome integrity by preventing instability, has also been shown to occur more frequently in the lung tumors of chromate-exposed workers compared to non-chromate lung tumors (Ali et al., 2011). APC suppression by mutation or CpG island hypermethylation is present in over 85% of colorectal cancers (Zhu et al., 2021).

P53 is a tumor-suppressor that normally regulates cell cycle arrest and apoptosis to protect against tumor formation; the induction of p53 target genes can indicate the presence of DNA damage, and inactivation of p53 is associated with carcinogenesis (Williams and Schumacher, 2016). P53 gene expression and protein levels were suppressed in the stomach (gene expression ≥3.5 mg/kg-day and protein levels ≥1.7 mg/kg-day Cr(VI)) and colon (gene expression and protein levels ≥5.2 mg/kg-day Cr(VI)) of male Wistar rats after 60 days of exposure to Cr(VI) in drinking water (Tsao et al., 2011). No studies of p53 expression in human GI tissues or nonneoplastic lung tissues are available, but studies in lung tumor tissues from chromate exposed vs. non-chromate exposed workers detected either no difference (Katabami et al., 2000) or increases (Halasova et al., 2010) in p53 protein expression, or reduced levels of p53 mutations (Kondo et al., 1997), and two

studies of the peripheral blood of exposed workers detected increased p53 protein expression (Elhosary et al., 2014; Hanaoka et al., 1997). However, although these studies in humans were not evaluated for risk of bias and sensitivity, little information was given regarding potential co-exposures, making it difficult to draw conclusions from these findings. In vitro, some studies show p53 activation in human lung cells increased with higher Cr(VI) concentrations (Hu et al., 2016) or occurring in vitro and not in vivo (Rager et al., 2017), so the nature of how p53 expression may be affected by Cr(VI) is not understood.

The oncogene c-Myc has also been shown to be differentially methylated in response to Cr(VI). Myc was found to show a dose-dependent increase (protein and mRNA) in the stomach and colon of male Wistar rats after 60 days of exposure in drinking water to Cr(VI) in the stomach (≥3.5 mg/kg-d) and colon (≥1.7 mg/kg-d) (Tsao et al., 2011). In context, these findings are consistent with the other observed effects of Cr(VI) exposure given the activity of this broad ranging oncogene, whose transcriptional control overlaps pathways of DNA damage response, cell proliferation and metabolism. Myc can be activated by another oncogenic pathway, the Wnt/β-catenin signaling pathway. Although no studies were identified that specifically investigated this pathway following Cr(VI) exposure, its involvement has been indirectly implicated by studies of Cr(VI)-induced methylation and subsequent downregulation of APC, a Wnt antagonist, as well as by the downregulation of serine/threonine kinase 11 and depletion of the Gene 33 protein (Lu et al., 2018; Li et al., 2017; Park et al., 2017).

An analysis of the toxicogenomic data reported in Kopec et al. (2012b; 2012a) from mice exposed to Cr(VI) in drinking water has identified a potential role for CFTR (cystic fibrosis transmembrane conductance regulator) in the carcinogenic effects of Cr(VI) (Mezencev and Auerbach, 2021). A tumor suppressor function has been demonstrated for CFTR in the GI tract of *Cftr* knockout mice (Than et al., 2016). *Cftr* gene expression was decreased in mice exposed to Cr(VI) levels as low as 0.1 mg/L Cr(VI) (0.024 mg/kg-d) in drinking water for 8 days. Loss of CFTR expression in humans was found to correlate with the severity of colorectal cancer, and in animals with a mutated *Apc* gene, to potentiate tumor progression Than et al. (2016). Although this effect has not been characterized beyond this single analysis, the implications of a specific Cr(VI)-induced CFTR suppression contributing to cancer risk in humans warrants further investigation.

Genomic instability (KC#3)

Genomic instability, an increased rate in the acquisition of genomic alterations, is an enabling characteristic of cancer and is present in nearly all human cancers (Hanahan and Weinberg, 2011; Negrini et al., 2010). As mentioned above, Cr(VI) exposure induces the suppression of DNA repair genes involved in mismatch repair. Defective mismatch repair leads to a form of genomic instability, microsatellite instability, which is a state of genetic hypermutability that is closely associated with colorectal cancer in humans (Boland and Goel, 2010). Microsatellite instability has been detected in the lung tumors of chromate workers compared to non-chromate

workers (Hirose et al., 2002), suggesting that Cr(VI) exposure may facilitate increased genomic instability, and ultimately cancer initiation and progression.

In addition to microsatellite instability, Cr(VI) exposure is also associated with increased aneuploidy, a numerical chromosomal aberrations that involves chromosome malsegregation and breakage (Eastmond et al., 2009) that is endemic of chromosomal instability and is a hallmark of cancer (Ben-David and Amon, 2020). Several studies have shown the ability of Cr(VI) to induce aneuploidy in vitro, summarized in Appendix Table C-54 and by Wise and Wise (2010). Aneuploidy was confirmed by detection in kinetochore-positive micronuclei (Güerci et al. (2000), Seoane et al. (2002; 2001, 1999)) or by chromosome painting with fluorescent probes (Figgitt et al., 2010). Exogenous agents inducing aneuploidy may act by interfering with the mitotic spindle apparatus via disruption of the microtubule cytoskeleton, a mechanism that is consistent with studies of Cr(VI)-induced aneuploidy (Nijs and Kirsch-Volders (1986), Seoane et al. (2002; 2001, 1999)). It is also plausible that altered DNA damage and repair pathways (e.g., loss of functional p53 and activation of driver oncogenes like Myc, reviewed above) can increase aneuploidy by promoting cell cycle progression before repair pathways can be initiated, resulting in chromosome malsegregation. APC, a tumor-suppressor gene associated with colorectal cancer when suppressed via promoter hypermethylation or mutation, has also been shown to have a key role in mitotic spindle orientation (Juanes, 2020). Although the mechanism for induction of aneuploidy by Cr(VI) is not known, the APC gene was found to be silenced by hypermethylation in the lung tumors of chromate-exposed workers (Ali et al., 2011), providing a hypothesis for how aneuploidy may be induced by Cr(VI), disrupting cell division and contributing to carcinogenesis; further research is warranted.

Gene and chromosomal mutation (KC#2)

The evidence for the genotoxic effects of Cr(VI) is presented and synthesized in Section 3.2.3.3. There is consistent and coherent evidence that a mutagenic MOA for Cr(VI)-induced carcinogenesis is biologically plausible and relevant to humans. Primary evidence is provided by *medium* and *low* confidence studies of occupationally exposed humans; some evidence is available in animals exposed directly in the lung or GI tract, but this evidence base is small and consists of *low* confidence studies, many of which were not optimized for reliably detecting genotoxicity. Genotoxicity studies employing more direct exposures to Cr(VI) (e.g., in vitro and in animals exposed via i.p. injection) are largely positive (summarized in Appendix C.3.3.3 and C.3.3.4), consistent with the intracellular pharmacokinetics and DNA reactivity of Cr(VI), as discussed above.

Suppression of apoptosis (KC#10)

The ability to resist cell death is a hallmark of cancer, contributing to the fixation of mutations and unchecked cell proliferation (Hanahan and Weinberg, 2011). Although initial exposures to Cr(VI) induce cytotoxicity (see below), there is evidence from one study of longer duration exposures that Cr(VI) can lead to the downstream suppression of programmed cell death

via apoptosis in tumor target tissues. Tsao et al. (2011) measured protein and mRNA levels in the stomach and colon of male rats following 60-day exposures to Cr(VI) in drinking water and reported decreased expression of RKIP, a MAPK inhibitor, and activation of the MEK/ERK signaling pathways, which promotes cell proliferation and inhibits apoptosis (Guo et al., 2020). In addition, the same study found decreased expression of p53 (gene and protein), the mediator of a primary cellular fate determination pathway, which would also lead to suppression of apoptosis (Tsao et al., 2011). This suggests a possible mechanism for a Cr(VI)-specific suppression of apoptosis via disruption of p53-mediated pathways that respond to cellular stress, although this is an area that requires further investigation.

Cytotoxicity and degenerative cellular changes (KC#10)

Cr(VI), a strong oxidizer, is known to be cytotoxic in vitro and may trigger apoptosis through increased oxidative stress, leading to DNA and protein damage, mitochondrial dysfunction, and modulation of pro-apoptotic signaling pathways. The reduction of Cr(VI) generates reactive intermediates Cr(V) and Cr(IV) that produce reactive oxygen species that can lead to apoptosis and necrosis, as well as induce cell signaling pathways associated with cell death (reviewed in Levina and Lay (2005) and Shi et al. (2004)). Because this evidence is relevant to both cancer and noncancer mechanisms of toxicity, these effects are reviewed in Sections 3.2.3.1 and 3.2.3.2 for the lung and GI tract, respectively. To summarize, this evidence supports a toxicity pathway of tissue injury induced by cytotoxicity in the lung and GI tract that may lead to necrosis and/or regenerative proliferation. In the lung, studies investigating the underlying mechanisms involved in Cr(VI)-induced lung toxicity report significant cytotoxicity at micromolar concentrations in vitro, concurrent with indications of increased programmed cell death (apoptosis, autophagy) in response to Cr(VI) exposure. In the GI tract, evidence of GI tract toxicity that involves Cr(VI)-induced cytotoxicity and apoptosis leading to degenerative changes and regenerative hyperplasia, as well as cell proliferation directly induced by Cr(VI). Other evidence of gene expression changes indicate cell signaling pathways induced by Cr(VI) exposure that are involved in the evasion of apoptosis contributing to tumorigenesis, indicating a downstream role independent of the cytotoxic effects of Cr(VI) that separately contributes to carcinogenesis by suppressing apoptosis. These cellular and molecular processes underlie the histopathological changes, including hyperplasia of the small intestine (described in Animal Evidence), that are considered potentially preneoplastic events.

Cell proliferation (KC#10)

Cancer is the result of sustained and uninhibited cell proliferation (Hanahan and Weinberg, 2011). Several studies have identified proliferative markers and signaling pathways that are upregulated by Cr(VI) exposure. Increases in transcript expression of Ki-67, a nuclear protein associated with cellular proliferation, and in some cases malignant metastasis and tumor growth (Li et al., 2015a), was detected in the duodenum of mice after exposure to 11.6 and 31 mg/kg Cr(VI)-

day in drinking water; levels were increased approximately 4-fold after 7 days of exposure but diminished to approximately 2-fold after 90 days (data from Kopec et al. (2012a) was presented graphically in Thompson et al. (2013)). In another drinking water exposure study, a dose-dependent upregulation of the *c-Myc* oncogene was found in the stomach (≥3.5 mg/kg-d) and colon (≥1.7 mg/kg-d) of male Wistar rats after 60 days of exposure to Cr(VI) in drinking water (Tsao et al., 2011). MYC functions as a transcription factor that upregulates genes involved in cell proliferation and other processes contributing to neoplastic transformation (Gabay et al., 2014).

Another transcription factor, AP-1, was found to be significantly activated by Cr(VI) exposure in studies of gene expression changes in human lung cells (Zuo et al., 2012; O'Hara et al., 2004) and in human breast cancer and rat hepatoma cells (Kaltreider et al., 1999). The AP-1 complex, which is composed of oncogenic proteins (Jun, Fos, ATF, MAF) (Eferl and Wagner, 2003), is induced by JNK and ERK/MAPK signaling cascades in response to stress and inflammatory cytokines (Gazon et al., 2017), leading to increased cell proliferation and/or inhibition of apoptosis, in part through the activation of cyclin D1 (Guo et al., 2020). Cyclin D1, a regulator and promoter of cell cycle progression, has been detected at significantly increased levels in the lung tumor tissues of chromate-exposed patients compared to unexposed lung cancer patients (Katabami et al., 2000). Increased expression of cyclin D1 has been associated with cell proliferation and tumorigenesis (Guo et al., 2020). These findings are consistent with an induction of biological processes by Cr(VI) that can lead to sustained cell proliferation and contribute to cancer. It is currently unknown to what extent these proliferation-promoting pathways are initiated by Cr(VI)-induced epigenetic repression of transcriptional regulators or are the result of a compensatory response to cytotoxicity and DNA damage sensing and repair machinery (discussed below), or if other direct or indirect factors induced by Cr(VI) are involved.

Regenerative hyperplasia

Hyperplasia is the enlargement of a tissue or organ resulting from increased cell proliferation and can be induced as an adaptive or compensatory response to cellular and tissue damage. In the evaluation of noncancer effects in the GI tract from ingested Cr(VI), hyperplasia is considered to be an adverse effect (Section 3.2.3), but it can also represent preneoplastic lesions that are part of the morphologic and biologic continuum leading to cancer (Hanahan and Weinberg, 2011; Boorman et al., 2003). Because hyperplasia can also be a reversible effect, it is important to consider several relevant factors when determining the contribution of hyperplasia to tumorigenesis, including whether there is a common cellular origin for hyperplasia and tumors, the presence or absence of a morphological continuum within the study between hyperplasia and neoplasia, histologic similarities, whether there is treatment-related toxicity, and other information about the test compound, including mutagenicity and ADME considerations (Boorman et al., 2003).

The diffuse intestinal epithelial hyperplasia observed in mice across studies is described in detail in Section 3.2.2.2. In the NTP (2008) 2-year bioassay, minimal to mild diffuse hyperplasia was significantly increased in the duodenum of all exposed male and female mice. These animals also

exhibited tumors of epithelial origin (adenomas and carcinomas) that were statistically significant at the two highest exposures (≥2.4 mg/kg-d in males, ≥3.2 mg/kg-d in females) with a dose-response trend in lower dose groups. There were multiple shared pathological features between the diffuse hyperplasia and the neoplastic lesions, including elongated crypts with increased numbers of epithelial cells and mitotic figures (NTP, 2008). These observations are generally consistent with the intestinal hyperplasia observed in mice in subchronic studies by NTP (2007) and Thompson et al. (2015a; 2011), lending further evidence of a consistent response in animals exposed to Cr(VI) via drinking water.

However, even with the presence of these morphologic similarities, in the absence of experiments with recovery groups to distinguish these lesions from reversible hyperplasia induced by Cr(VI), it cannot be concluded with certainty that the hyperplasia observed in the subchronic studies would have progressed to neoplasia. As discussed in Section 3.2.2.3, some discrepancies have been noted, including the lack of increased mitotic activity in hyperplastic duodenal crypt cells in mice (Thompson et al., 2015b; O'Brien et al., 2013), although follow-up analysis of the mice exposed via drinking water for 7 and 90 days (Thompson et al., 2011) reported a significant response in gene expression changes related to cell cycle progression in duodenal crypts at doses ≥4.6 mg Cr(VI)/kg-d (Chappell et al., In Press). In addition, as discussed above, although Thompson et al. (2013) reported levels of the cellular replication marker Ki-67 were increased compared to untreated controls in mice exposed for 7 or 90 days in drinking water, these levels declined in the mice exposed for 90 days, and Ki-67 cannot distinguish between chemically induced cell proliferation and proliferation secondary to cellular toxicity without concurrent detection of cellular markers for apoptosis and necrosis.

The presence of tissue injury is also important in interpreting the relevance of these lesions to neoplasia. Tissue-specific hyperplasia and neoplasia with an inciting factor such as cellular degeneration and compensatory regeneration may suggest a carcinogenic response that is secondary to chronic tissue injury (Boorman et al., 2003). As reviewed in Section 3.2.2.2, the authors of both sets of studies (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007) considered the hyperplastic lesions to be consistent with regenerative hyperplasia resulting from Cr(VI)-induced epithelial damage and degenerative changes seen in the mouse villi. This suggests a mechanism in the carcinogenic process that may be secondary to chronic tissue injury.

In addition to the diffuse hyperplasia, a non-statistically significant incidence of focal epithelial hyperplasia was observed in male mice at ≥2.4 mg/kg Cr(VI)-day that increased slightly in severity grading (3.0−3.5) with dose. Female mice also showed a low incidence of focal hyperplasia with increasing severity grading (2.0−3.0) at 1.2 and 3.2 mg/kg Cr(VI)-day with no reported incidences at the high dose (NTP, 2008). NTP considered the focal hyperplasia to be biologically significant preneoplastic lesions due to the pathological similarities to neoplastic growths, including crypts and villi that were lined by increased numbers of cuboidal to tall columnar epithelial cells that were morphologically similar to those of the adenomas (Francke and

Mog, 2021). In addition, these lesions, located in the superficial mucosa rather than the crypt mucosa, arose from the same tissue type (duodenal epithelium) as the neoplastic growths[31]. The focal hyperplastic lesions were distinguished from adenomas by their smaller size and less discrete margins that tended to blend with the normal surrounding mucosal epithelium.

While diffuse hyperplasia may have an origin in a regenerative response that is secondary to chemically induced tissue degeneration, focal hyperplasia that is morphologically similar to neoplasia without evidence of concurrent tissue injury may be indicative of a direct neoplastic response (Boorman et al., 2003). Although the focal hyperplasia could be a part of the proliferative continuum of lesions, progressing from diffuse hyperplasia to focal hyperplasia (preneoplastic), to adenoma (autonomous growth), to carcinoma (malignant neoplasia) originating from a common precursor cell type, this cannot be confirmed due to the absence of histopathological observations from interim sacrifices.

Thompson et al. (2012b) also reported duodenal hyperplasia and villous apoptosis in rats treated with ≥7.2 mg Cr(VI)/kg-d in drinking water for 7 and 90 days, as well as villous atrophy at 7.2 mg Cr(VI)/kg-d. Rats were not observed to develop intestinal lesions or tumors in the bioassays by NTP (2008, 2007). Rats developed tumors in the oral cavity, but there were no observations of lesions or hyperplasia in the rat oral cavity by any of these studies.

Hyperplasia has also been observed in the rat lung following inhalation exposures to Cr(VI) for 30 and 90 days (Glaser et al. (1990), see Section 3.2.1.2). A high incidence of bronchioalveolar hyperplasia (70–100%) was reported in male Wistar rats after 30 days of exposure to 0.050–0.40 mg/m^3 Cr(VI) relative to the control (10%) (Glaser et al., 1990). The same study reported lower incidence of this effect after 90 days of exposure, and after 90 days of exposure with a 30-day recovery period, suggesting this may have been a transient effect.

Overall, there is evidence for regenerative hyperplasia as a key event for tumors of the small intestine in mice. Theoretically, any increase in the rate of cell proliferation over the background basal rate of cell division, even if transient, can increase the probability of the formation and fixation of mutations that may confer a selective advantage to the cell and promote the subsequent clonal outgrowth of the mutated cells, leading to tumorigenesis. There are some inconsistencies that create uncertainty in drawing a conclusion that Cr(VI)-induced regenerative hyperplasia is a primary event driving carcinogenesis, including hyperplastic responses that did not increase in severity with dose, and the presence of degenerative lesions and hyperplasia in the rat small intestine with no induction of tumors at this site in this species. Regenerative hyperplasia may be a contributing factor to carcinogenicity in the lung, as toxicity and hyperplasia have been observed in the lung following inhalation exposures, though there is not enough evidence to assume a key role in this tissue. There is no evidence to conclude regenerative hyperplasia is involved in the tumorigenic process in the rat oral cavity.

[31]Most (76%) tumor-bearing animals were observed to have exhibited nonneoplastic lesions in the small intestine (see Appendix D.5).

Chronic inflammation (KC#6)

Cr(VI) has been shown to induce effects consistent with an inflammatory response by generating oxidative stress that can stimulate pro-inflammatory cytokines and activate nuclear transcription factors associated with inflammation (e.g., NF-κB). The evaluation of evidence for effects of Cr(VI) on the immune system, presented in Section 3.2.6, suggests that Cr(VI) may have a stimulatory effect on the immune system, largely based on primary immune response assays indicating increased antibody responses, WBC function and numbers, and total immunoglobulin levels following Cr(VI) exposure in animals (see Section 3.2.6). Although exposure-related stimulation of the immune system can lead to exaggerated inflammatory responses associated with chronic systemic inflammation, the role of inflammation in the carcinogenesis of the GI tract induced by Cr(VI) exposure (Section 3.2.2) is not clear.

The GI tract contains the majority of immunoglobulin-producing cells that are present in the human body, and toxicity to the GI tract commonly results in immune system-mediated inflammation (Gelberg, 2018). Chronic inflammation could have driven the diffuse hyperplasia observed prior to carcinogenesis in the mouse small intestine in the NTP subchronic and chronic bioassays, as this is a well-characterized step in inflammatory neoplastic progression, and is an enabling characteristic of cancer (Hanahan and Weinberg, 2011; Westbrook et al., 2010). The development of idiopathic GI cancers has been shown to involve chronic inflammation that can induce neoplastic genetic and epigenetic changes mediated by proinflammatory cytokines and ROS (Chiba et al., 2012). In addition, immunogenomic profiling of data from over 10,000 tumors collected by the Cancer Genome Atlas used cluster analysis to identify six immune subtypes commonly associated across multiple tumor types; one identified immune subtype, “wound healing," was associated with colorectal cancer, lung squamous cell carcinomas, head and neck squamous cell carcinomas, and the chromosomal instability (CIN) pathway of colorectal cancer pathogenesis (CGARN, 2018b), tumor tissues also associated with Cr(VI)-induced cancer. However, NTP reported that the rat oral cavity had neither hyperplasia nor inflammation preceding tumor formation, and no signs of inflammation were observed in the mouse small intestine after two years of drinking water exposure to Cr(VI). NTP did report an increased infiltration of histiocytes (macrophage immune cells) in the duodenum and jejunum that was consistently observed in both sexes of rats and mice orally exposed both chronically and subchronically to Cr(VI) (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007). However, this was not accompanied by an influx of other inflammatory cells or other histological features consistent with inflammation in the small intestine and was interpreted by the authors to be of unknown biological significance.

Evidence following inhalation exposures to Cr(VI) is more robust, with consistent evidence of histiocytosis in the lung from several studies in animals accompanied by inflammatory markers in BALF and increased leukocytes in plasma, observations supportive of inflammatory lung responses (Section 3.2.1). The histiocytic/macrophage infiltration leads to cytokine release and cell to cell signaling conducive to an inflammatory environment (Kodavanti, 2014). Studies

investigating immune toxicity (Section 3.2.6) in chromate workers have also observed changes in cytokine signaling (Appendix C.2.5.2). Although the direction of these changes was not consistent across studies, fluctuations in systemic cytokine levels and increased oxidative stress are characteristic of an inflammatory response and may indicate a disruption in the regulatory balance that dictates normal immune system function. However, while there is evidence of oxidative stress and activation of pro-inflammatory cytokines and nuclear transcription factors including NF-kB, the characterization of chronic inflammation that may occur prior to the development of neoplasms induced by Cr(VI) exposure remains an evidence gap.

Tumor formation

Neoplastic effects were not observed in subchronic 13-week studies in mice and rats (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2007), though notably some of the observations in the subchronic studies, including elongated intestinal crypts and increased mitotic activity, were also reported in the histopathological analysis of adenomas and carcinomas in the 2-year bioassay. The lack of tumor formation in the subchronic experiments is likely due to insufficient latency time. The earliest appearance of tumors of the mouse small intestine reported by NTP in the two-year bioassay (NTP, 2008) was at 451 days in males and at 625 days in females exposed to the highest tested Cr(VI) doses (5.7 mg/kg-d and 8.9 mg/kg-d in males and females, respectively). In all other dose groups, tumors in the mouse small intestine were reported at terminal sacrifice (729 days). The earliest recorded incidences of tumors of the rat oral cavity reported by NTP (2008) were at 506 days in females and at 543 days in males exposed to the highest tested Cr(VI) doses (7.1 mg/kg-d and 6.1 mg/kg-d in females and males, respectively). Several models have been proposed for the histopathogenesis of GI cancers that are potentially relevant to Cr(VI). One example is the classical model of transformation and clonal expansion of rapidly dividing, self-renewing stem cells at the bottom of the intestinal crypts, or the 'bottom up' model (Schwitalla et al., 2013; Shih et al., 2001; Bach et al., 2000). Alternatively, a 'top down' model of adenoma morphogenesis in a transgenic c-Myc mouse model system suggests that dysplastic cells at the luminal surface of the crypts have the ability to dedifferentiate and spread laterally and downward, forming new crypt-like foci (Schwitalla et al., 2013). This type of cellular phenotypic plasticity driven by oncogenic signaling, observed in colon cancers, is considered a hallmark capability of cancer (Hanahan, 2022). Expression of c-Myc also increases in the stomach and colon of rats after subchronic oral Cr(VI) exposure (Tsao et al., 2011), and toxicogenomic data demonstrate comprehensive activation of the c-Myc pathway and concurrent changes in known downstream target genes (Rager et al., 2017; Kopec et al., 2012b; Kopec et al., 2012a; Thompson et al., 2011). The dysplastic cells at the luminal surface are stem-like, preneoplastic, and represent mutant clones containing genetic alterations not found in the morphologically normal cells at the bottom of the crypt (Shih et al., 2001). This model is based in part on the frequent observation that early adenomatous polyps are found at the top of colonic crypts without stem cell compartment contact (Shih et al., 2001). Mechanistically, Schwitalla et al. (2013) proposed that NF-kB can

enhance Wnt signaling leading to dedifferentiation of epithelial non-stem villus cells into tumor-initiating cells. In addition, the cell proliferation marker Ki-67, which was increased in the duodena of mice after exposure to Cr(VI) in drinking water (Rager et al., 2017; Kopec et al., 2012a), has been shown to be increased in the dysplastic crypt orifices of idiopathic human intestinal adenomas (Shih et al., 2001).

Evidence favoring the 'bottom up' model is provided by a follow-up analysis of the mice exposed via drinking water for 7 and 90 days (Thompson et al., 2011), which determined that a robust response in gene expression changes was present in the crypts at doses ≥4.6 mg Cr(VI)/kg-d, and that the enrichment of gene sets related to cell cycle progression and DNA damage were more robust in the crypts compared to the villi (Chappell et al., In Press). Alternatively, there is evidence for the 'top-down' model, as X-ray fluorescence microspectroscopy in a separate study by this group detected a 35-fold higher mean Cr(VI) concentration in the villi compared to the intestinal crypts (Thompson et al., 2015a). The precise mechanism for how Cr(VI) would initiate a 'top-down' process is unknown but could plausibly involve mutagenic processes. Although inconclusive due to incomplete reporting, O'Brien et al. (2013) reported increased micronucleus frequency in the duodenal villi of Cr(VI)-exposed mice, suggesting that further investigation is warranted. Neither model can be reliably ruled out without further investigation.

There is considerable uncertainty regarding the origin of the tumors observed in the rat oral cavity by NTP (2008). A recent review of chemicals that have been shown to cause oral squamous cell neoplasms by the NTP suggests multiple mechanisms can promote rat oral tumors (Ibrahim et al., 2021). An i.p. injection experiment in female Wistar rats showed effects on the submandibular gland which may support the findings of oral cancer in rats. Submandibular acinar saliva-secreting cells showed an increase in cystatin staining, which may play a role in tumorigenesis, metastasis, and immunomodulation (Ochieng and Chaudhuri, 2010; Cohen et al., 1993). Inducible type 2 cystatin was not detected in the parotid or sublingual glands, trachea, lung, stomach, small intestine, large intestine, spleen, liver or pancreas, suggesting that Cr(VI)-induced effects on cystatins are likely to be localized.

Cancer mode-of-action summary

The mechanistic events identified above have some level of Cr(VI)-specific evidence to indicate their involvement in the carcinogenic effects of Cr(VI). These events are biologically plausible in that they are known to be associated with carcinogenesis and can occur in humans, with interrelated pathways that emerge involving mutagenicity, cytotoxicity and regenerative cellular proliferation. The molecular events involved in these effects are assumed to be relevant to all routes of exposure. The evidence-based assumption is that some amount of unreduced Cr(VI) can reach target tissues when ingested or inhaled and can be quickly taken up by the cells in these tissues, where it will be reduced intracellularly to reactive intermediates that induce toxic and carcinogenic effects. At the tissue level, differences in the evidence for each tumor type also emerge, therefore it is unclear whether some mechanistic events are key for every tumor, as the mechanistic

effects may be dependent on the specific pattern or duration of activation of certain events. These may occur based on cell type-specific properties such as their baseline proliferative rate or ability to mitigate the effects of oxidative stress. Cr(VI) is a known human lung carcinogen, therefore specifics of lung tumors will not be discussed here in the context of mechanistic evidence, but the mechanistic evidence from studies of the exposed lung is considered relevant and discussed along with mechanistic evidence for the tumors of the mouse small intestine. There is a lack of empirical mechanistic evidence from the rat oral cavity.

There is extensive evidence of the mutagenicity of Cr(VI) when reduced intracellularly to Cr(III) in studies conducted among in vitro test systems. A mutagenic MOA is also supported in test animals when considering the evidence in the context of pharmacokinetic considerations. Although the evidence of mutation from oral exposures is less consistent, the genotoxicity observed in animal i.p. studies in vivo has been consistently observed. Therefore, evidence of transmissible and permanent genetic alterations have been prioritized for the analysis of a mutagenic MOA if observed following oral or inhalation exposures in GI or lung tissues.

Cr(VI) is a known lung carcinogen, and a mutagenic MOA is supported for lung tumors following inhalation exposures primarily by evidence of increased micronuclei detected in the blood and exfoliated nasal and oral epithelial cells from occupationally exposed humans. Mutagenic activity also correlates with blood chromium levels in *medium* confidence studies, and several *low* confidence human studies that demonstrate increased chromosomal aberrations despite many having limitations that would potentially lead to bias toward the null. Supporting evidence is also provided by studies showing increased levels of DNA damage in exposed workers, as well as one *low* confidence study of mutations in the mouse lung that increased with dose and time following intratracheal instillation, providing biological plausibility that mutation is involved in the development of Cr(VI)-induced lung cancers. Therefore, a mutagenic MOA for lung tumors is considered to be relevant to humans and sufficiently supported in laboratory animals after inhalation exposure, based on the following: 1) the evidence-based interpretation that some amount of inhaled Cr(VI) (at physiologically relevant doses) escapes detoxification and is taken up by target cells; 2) this uptake is expected to occur more readily in regions of the lung showing a high chromate deposition that correlate with sites of lung tumors in exposed workers; 3) demonstrations of increased chromosomal mutations in the exfoliated nasal and buccal cells and in the peripheral blood of occupationally exposed workers; 4) gene mutations in the mouse lung that increased with dose and time post-intratracheal instillation; 5) other genotoxic effects in the peripheral blood of exposed workers and in lung-derived cell cultures in vitro; and 6) mutagenicity of Cr(VI) when it reaches cells of various tissue types in vivo and in vitro. The implications of a mutagenic MOA for the dose-response analysis and inhalation unit risk calculation for lung cancer are presented in Section 4.4.3.

The evidence for a mutagenic MOA following oral exposures is less clear. There are no human oral exposure studies of mutation in the GI tract, although consistent evidence of increased

micronucleus frequency in the oral epithelial cells of exposed workers may support the evidence that Cr(VI) can induce mutagenic effects when it comes into contact with cells in the GI tract, and contributes to an evaluation of whether mutation may be a primary neoplastic event. The database of in vivo oral animal exposure genotoxicity studies that are specific to GI tissues is limited to a small number of *low* confidence studies, most of which have deficiencies in sensitivity for detecting an effect or other concerns that introduce a large amount of uncertainty.

The mutagenicity assays used by these studies were originally designed and optimized for purposes of identifying hazard, namely, whether a chemical is capable of inducing increased mutagenic damage, regardless of dose. Although several doses are typically employed, these assays are not optimized for dose response, and typically use a minimal number of animals (1-5). Therefore, it is important that these assays use a range of doses that include a maximum tolerated dose (MTD) or otherwise indicate that the chemical reached the target tissue to ensure sensitivity (Hayashi, 2016) and that null findings represent a true lack of effect (versus a deficiency in study design). As with all genotoxicity assays, these tests are often considered in an MOA analysis for cancer, with the hypothesis that evidence of mutation in the tumor target tissue occurs earlier than the induction of tumors, in the same species, and at the same doses causing tumors supports a mutagenic MOA. Evaluations of this hypothesis often presume the converse also applies, in that a negative result will indicate a lack of mutagenicity and therefore support an alternate MOA that does not involve mutagenicity. This assumption often relies on testing results within an acute to subchronic exposure period in a small number of animals. It is difficult to make a definitive conclusion that Cr(VI) is not mutagenic in the GI tract following oral exposures from an evidence base in animals composed of mostly null results from a small number of *low* confidence studies, given that Cr(VI) has been shown to be mutagenic in other exposures and test systems, and we can reasonably expect that ingested Cr(VI) will reach the GI tract.

High levels of cytotoxicity can lead to the detection of increased DNA damage in some test systems. For this reason, the interpretation of genotoxicity evidence from chemicals inducing excessive toxicity includes efforts to determine whether increases in genotoxicity are potentially secondary to cytotoxicity. For the Cr(VI) in vivo oral exposure database, there is not enough evidence to determine whether and to what extent Cr(VI)-induced genotoxicity might be the result of (secondary) cytotoxic DNA damage in the GI tract. Most notably, while many of the animal studies examining the most relevant genotoxicity endpoints did not detect substantial evidence of genotoxicity at doses that also caused histological effects in the GI tract, including diffuse epithelial/crypt cell hyperplasia and degenerative changes in the villi (vacuolization, atrophy, and apoptosis), one study did observe statistically significantly increased micronuclei in villous cells from animals exposed to doses that similarly induced villous atrophy and apoptosis. Because no studies were available that specifically examine the presence or absence of genotoxicity in the GI tract as the MTD was approached and exceeded, this uncertainty cannot currently be addressed.

Although it is presumed that ingested Cr(VI) can reach the target tissues in at least a fraction of humans and animals, there are pharmacokinetic differences between oral and inhalation exposure routes that indicate lower concentrations of Cr(VI) will reach target tissues when ingested than when inhaled. In this context, however, it is still not possible to conclude that there is no potential risk of increased mutations occurring in humans ingesting Cr(VI) in drinking water, particularly when taking into consideration human subpopulations with a diminished ability to reduce Cr(VI) in the stomach due to low gastric pH (see 'Susceptible populations' in the following section). Therefore, a mutagenic MOA is supported for GI tumors after oral exposure, based on the following: 1) the evidence-based interpretation that some amount of ingested Cr(VI) (at physiologically relevant doses) escapes GI detoxification and reaches target cells; 2) the demonstrated chromosomal mutations in buccal cells of occupationally exposed workers; 3) the demonstrated mutagenicity of Cr(VI) when it comes into direct contact with any cell type in various tissues in vivo and in vitro; and 4) a lack of studies designed to adequately test for mutagenicity in the target tissue after ingestion exposure.

The mutagenic effects of Cr(VI) in the lung and GI tract are expected to be amplified by promutagenic effects that are also anticipated to be key events for cancer induced by Cr(VI). Oxidative stress induced by reactive Cr(VI) intermediates can damage DNA and intracellular proteins and lead to an imbalance between free radicals and antioxidants. Direct and indirect suppression of DNA repair processes via epigenetic silencing may lead to increased DNA damage, DNA double-strand breaks, and genomic instability including microsatellite instability and aneuploidy. The epigenetic modifications induced by Cr(VI) include extensive promoter-specific hypermethylation, global hypomethylation, post-translational histone modifications, and microRNA dysregulation. These perturbations can affect the expression of an extensive number of genes including tumor suppressors and oncogenes associated with lung and colorectal cancers that involve the promotion of unchecked cellular proliferation along with the suppression of apoptosis. Although epigenetic changes are not permanent changes to the gene sequence, their overall effect can be analogous to mutation in that they are heritable changes affecting gene expression. The oxidative stress, oxidative DNA damage, direct or epigenetic suppression of DNA repair processes, and genomic instability induced by Cr(VI) are all likely to be key events for carcinogenesis applicable to oral and inhalation exposures for all tumor types. These effects combine to produce a promutagenic microenvironment that promotes the formation and fixation of mutations from DNA damage, regardless of whether the genetic damage was produced endogenously, by Cr(VI), or from another source.

Consistent evidence of an inflammatory response in the lung following inhalation Cr(VI) exposures in animals indicates this effect is likely to be a key event for lung cancer. Although idiopathic cancer development in the GI tract has also been shown to involve chronic inflammation (Chiba et al., 2012), no histopathological evidence of GI inflammation induced by Cr(VI) oral exposure was observed in animals exposed via drinking water. However, the inflammatory

response associated with GI tract cancers has been shown to be mediated by proinflammatory cytokines and ROS, effects that are known to result from Cr(VI) oral exposures and can lead to genetic and epigenetic changes that promote neoplastic transformation. Combined, these data suggest that inflammation could still be involved in the neoplastic effects of the small intestine in mice.

An alternative MOA for carcinogenicity induced by ingested Cr(VI) is regenerative proliferation caused by tissue injury, leading to a higher probability of spontaneous mutations that may result in tumorigenesis. Cr(VI), a strong oxidizer, is known to be cytotoxic in vitro and may trigger apoptosis through increased oxidative stress, mitochondrial dysfunction, and modulation of pro-apoptotic signaling pathways. Following oral exposures, regenerative hyperplasia interpreted to be the result of regressive changes such as villous blunting, villous atrophy, and apoptosis of enterocytes was consistently observed in the mouse small intestine (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007). Inconsistencies in the hyperplastic responses to these degenerative changes have been noted, however, including hyperplasia that did not increase in severity with dose, and no statistically significant or dose-responsive changes in mitotic or apoptotic indices in tissue regions where increased crypt length, area, and number of crypt enterocytes were reported. The diffuse hyperplasia of the small intestine is likely to be a key event for tumors in this tissue, although these hyperplastic lesions, which were also observed in the rat small intestine by Thompson et al. (2012b), do not always progress to cancer and can represent a functionally adverse change on their own.

The GI tract has a high capacity for tissue regeneration following cellular injury, which makes it more sensitive to exposures that may interfere with the process of cell division (Nolte et al., 2016). At least some of the molecular events affecting cell cycle regulation that are altered by Cr(VI) exposure also appear to underlie the regenerative histopathological changes in animals exposed to Cr(VI). A toxicogenomic analysis comparing gene expression changes in the duodenal crypts and villi of the mice exposed via drinking water for 7 and 90 days (Thompson et al., 2011) found a robust response in the crypts at doses ≥4.6 mg Cr(VI)/kg-d, and that the enrichment of gene sets related to cell cycle progression and DNA damage were more robust in the crypts compared to the villi (Chappell et al., In Press). Other toxicogenomic evidence consistent with increased cellular proliferation in the mouse small intestine, including increased expression of oncogenic c-Myc and the proliferative marker Ki-67, provides additional support for increased cell proliferation occurring in the preneoplastic small intestine, although these markers are not specific to regenerative hyperplasia. It is also not clear whether the degenerative and regenerative effects are key events for other tumor types. No lesions or hyperplasia have been reported in the rat oral cavity, and while cellular injury and hyperplasia were observed in the rat lung following inhalation exposures, the hyperplasia diminished with longer exposures and following a recovery period.

The focal hyperplasia observed only in the mouse small intestine, although not statistically significant or dose-dependent, represents a biologically important preneoplastic event that could

result from the interaction between Cr(VI)-induced regenerative processes and mutagenic effects (NTP, 2008). These lesions were observed closer to the hyperplastic villous region of the superficial intestinal mucosa, where Cr(VI) has been shown to concentrate (Thompson et al., 2015a). Some evidence of micronuclei and oncogenic transformation has also been observed in this tissue (O'Brien et al., 2013; Tsao et al., 2011). This indicates the potential for a combined MOA for Cr(VI)-induced tumorigenesis in the small intestine after oral exposure, where mutagenic effects occur concurrently with hyperplasia, providing an environment that can support the clonal expansion of mutated cells.

Although no histopathological changes were observed in the rat oral cavity preceding tumor formation in subchronic or chronic bioassays of Cr(VI) in drinking water, and no increases in mutation frequency were observed in these tissues in a single study investigating this endpoint, mutagenicity is a biologically plausible mechanism and is coherent with the evidence of increased micronuclei in the buccal cells of exposed humans. Although site concordance is not a requirement when considering the evidence for a mutagenic MOA, there is currently not an understanding of why humans do not show evidence of oral tumors, or why rats do not have tumors of the small intestine. It is plausible that extensive epigenetic alterations, which have been shown to account for phenotypic differences among individuals as well as among different tissue and cell types (Zhang et al., 2013), may influence the differences in carcinogenic response and the carcinogenic potency of Cr(VI) at the tissue level or even among individuals and across species.

In conclusion, the available mechanistic evidence supports key events at the molecular and cellular level that are expected to be applicable to all exposure types and tumors. These key events are summarized in Table 3-21 and Figures 3-16 and 3-18. Cr(VI) that is not reduced extracellularly may be taken up by cells near the point of contact, which is generally expected to be the lung for inhalation exposures and the GI tract for oral exposures. The GI tract, including the oral cavity, is expected to be exposed by both of these routes in humans (impaction of dusts in the mouth and tongue resulting from oral breathing and mucociliary clearance may result in GI exposure via the inhalation route). Oxidative stress occurs within the cell, generated by the reactive chromium intermediate species, inducing DNA damage and cytotoxicity. Chromium-DNA adducts can be formed by the ultimate Cr(III) species and, in combination with suppressed DNA repair processes via epigenetic modifications, these adducts and other oxidative DNA damage may be fixed as mutations in these cells. Cr(VI) may also promote aneuploidy and microsatellite instability by suppressing DNA mismatch repair. These promutagenic effects, combined with epigenetic modifications influencing the suppression of apoptosis and increased cell proliferation, combine to create a tumor microenvironment supporting the clonal outgrowth of mutated cells. In addition, there is evidence from the small intestine of mice exposed via drinking water that Cr(VI) exposure can induce degenerative effects at the tissue level, with a proliferative response that should promote the selection of cells with a growth advantage, leading to tumorigenesis, though it is

unclear whether this occurs in all tumor types. These processes also likely involve chronic inflammation, though there is inconsistent evidence of this in all tumor tissues.

Table 3-21. Evidence profile table for the carcinogenic mechanisms of inhaled or ingested Cr(VI)

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
Distribution of Cr(VI) (Sections 3.1.1 and 3.2.3.4; Appendix C.1.2)	*Lung*: • Inhaled Cr(VI) comes into direct contact with lung epithelial cells and is expected to be directly absorbed with minimal extracellular reduction (i.e., detoxification) due to a less favorable reduction environment in lung tissues • Cr(VI) accumulates at lung bifurcation sites in the lungs of chromate workers • Cr(VI) burden in the lung correlates with lung cancer incidence *Oral cavity*: Following inhalation or oral exposures, cellular uptake may occur in the epithelium of the oral mucosa, tongue, and esophagus (prior to Cr(VI) reduction in the stomach), although the surface area for mass transfer is low *Stomach*: While reduction (i.e., inactivation) of ingested Cr(VI) occurs in the stomach, it will compete with gastric emptying of Cr(VI) to the small intestine. Uptake in the stomach epithelium is also possible, although the surface area for mass transfer is low *Small intestine*: • Cr(VI) bioavailability and kinetic considerations suggest that 10–20% of ingested Cr(VI) escapes human gastric inactivation and could expose the GI tract epithelium • Cr(VI) exposure to the proximal small intestine will be greater than exposure to the distal small intestine, as the Cr(VI) concentration decreases • The surface area for mass transfer in the small intestine is high	Following exposure to Cr(VI), it has been demonstrated that inhaled Cr(VI) can reach cells in the lung and oral cavity, and after ingestion, Cr(VI) can reach cells in the oral cavity (either by movement through the GI tract after inhalation and deposition into the oral cavity, or by direct ingestion), stomach, and small intestine, both potentially in appreciable amounts to elicit an effect. Distribution is strongly dependent on route of exposure (inhalation à respiratory tract, oral ingestion à gastrointestinal tract and liver)
Cellular uptake of Cr(VI) (Sections 3.1.1 and 3.2.3.4; Appendix C.1.1)	*All cell types*: Cr(VI) is rapidly taken up by nonspecific sulfate and phosphate transporters due to the structural similarity of Cr(VI). *Lung*: Particulates may deposit and absorb locally; the amount taken up is dependent on location, particle size, and solubility. *Oral cavity*: Morphology within different regions of the oral cavity is highly variable (hard palate, buccal mucosa, gingiva, ventral/dorsal tongue, lip), and may impact localized cellular uptake. *Stomach*: Lower absorptive surface area and different morphology than the small intestine. Some uptake may occur prior to gastric emptying. *Small intestine*: Highly absorptive surface area increases uptake of Cr(VI) (primarily by the villi).	Ingested or inhaled Cr(VI) can be taken up by cells in tumor target tissues.

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
Intracellular reduction of Cr(VI) (Sections 3.1.1.3 and 3.2.3.4; Appendix C.3.2.1)	*All cell types*: Following cellular uptake, Cr(VI) is reduced primarily by ascorbate, but other biological reductants (e.g., cysteine, GSH) are also capable of reducing Cr(VI). This leads to the intracellular formation of the reactive intermedidate species Cr(V) and Cr(IV) and the stable Cr(III).	Intracellular reduction is considered an activation pathway, generating reactive intermediates capable of damaging DNA directly or indirectly via oxidative damage.
DNA reactivity (Section 3.2.3.4; Appendix C.3.2.1)	*All cell types:* Intracellular Cr(III) has been demonstrated to be DNA reactive and can form stable complexes with DNA, RNA, amino acids and proteins, including Cr(III)-DNA adducts, DNA-DNA crosslinks, and DNA-protein crosslinks.	Intracellular Cr(III) can bind to DNA, which can form bulky adducts that cause replication fork stalling, DNA double-strand breaks and mutations if not adequately repaired or eliminated by apoptosis.
Oxidative stress and oxidative DNA damage (Section 3.2.3.4; Appendix C.3.2.5)	*Inhalation exposure:* • Consistent evidence of significant increases in oxidative stress in workers exposed to Cr(VI) that correlated with levels of Cr(VI) in urine and blood (see Appendix C.2.1 and C.3.9) • Increased formation of 8-OHdG DNA adducts in rats exposed to Cr(VI) via inhalation (Maeng et al., 2003) or intratracheal instillation (Zhao et al., 2014; Izzotti et al., 1998) *Oral exposure:* • Decreased GSH/GSSG ratio in small intestinal epithelium after 7 and 90 days of oral dosing in mice and after 90 days in rats, and in oral mucosa in mice after 7 days and rats at 90 days, although no 8-OHdG adducts or protein oxidation in any tissues (Thompson et al., 2011; De Flora et al., 2008) • Activation of genes involved in oxidative stress in the duodenum of mice exposed to Cr(VI) for 90 days but not after 7 days *In vitro:* • Detection of reactive intermediates in acellular systems • Oxidative stress in human primary and immortalized lung or GI cells after exposure to Cr(VI), including increased ROS production, oxidation of lipids and proteins, and increased antioxidant enzyme activity • Increased intracellular reduction via ascorbate correlates with free radical production, oxidative DNA damage (e.g., 8-OHdG adducts, DNA strand breaks, DNA-protein crosslinks, alkali labile sites) and lipid peroxidation	A consistent and coherent evidence base shows redox reactions during intracellular reduction of Cr(VI) produce reactive oxygen species that cause DNA damage in occupationally exposed humans, experimental animal studies, and in vitro studies, although the evidence in animals exposed orally is less consistent

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
	• Addition of antioxidants reduces/eliminates oxidative DNA damage; suppression of antioxidants or use of DNA repair deficient cell line increases oxidative DNA damage • Dose-dependent activation of NF-kB and AP-1, pro-inflammatory transcription factors and redox-sensitive signaling molecules	
Epigenetic modifications (Section 3.2.3.4; Appendix C.3.2.4)	*Inhalation exposure:* • Hypermethylation of tumor-suppressor genes $P16^{ink4a}$ (Kondo et al., 2006) and APC (Ali et al., 2011) in chromate factory workers with lung cancer who had occupational chromate exposure compared to those without chromate exposure, and dysregulation of tumor suppressor microRNAs that correlate with Cr blood levels (Li et al., 2014b). • Hypermethylation of DNA mismatch repair and homologous recombination repair genes in lung cancer cases with chromate exposure (Hu et al., 2018; Ali et al., 2011; Takahashi et al., 2005), leading to microsatellite instability • Global hypomethylation in chromium-exposed workers (Linqing et al., 2016; Wang et al., 2012b) *In vitro:* Extensive evidence of the epigenetic mechanisms of Cr(VI) (including methylation, histone modifications, and miRNA) (reviewed in Chen et al. (2019)) and increased resistance to apoptosis in human colon cells lacking a key mismatch repair gene when exposed to Cr(VI)	Consistent, coherent evidence of epigenetic alterations (heritable changes in gene expression that are not caused by changes in DNA sequence) that correlate with Cr(VI) exposure in humans and are known to contribute to microsatellite instability, mutagenicity, and carcinogenesis.
Inhibition of DNA repair (Section 3.2.3.4; Appendix C.3.2.3)	*Inhalation exposure:* epigenetic suppression of genes involved in DNA repair in Cr(VI)-exposed workers (summarized above) *In vitro*: Inhibition of genes involved in mismatch repair (see above) and homologous recombination repair, including RAD51 (Browning et al., 2016; Hu et al., 2016; Li et al., 2016; Bryant et al., 2006)	Consistent, coherent evidence of the epigenetic suppression of DNA mismatch repair (see above) and homologous recombination repair, leading to increased DNA double-strand breaks that are more likely to cause mutations.
Genomic instability (Section 3.2.3.4; Appendix C.3.2.3)	*In vitro:* Consistent evidence of aneuploidy induced by Cr(VI) ((Figgitt et al., 2010), (Güerci et al., 2000), Seoane et al. (2002; 2001, 1999))	Besides the microsatellite instability induced by epigenetic suppression of DNA mismatch repair (see above), Cr(VI) may also cause aneuploidy, a hallmark of cancer. This evidence is primarily from in vitro studies.
Genotoxicity and mutagenicity	*Inhalation exposure:*	Consistent observations of heritable structural and numerical genetic damage in

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
(Section 3.2.3.3; Appendix C.3.2.2)	• Consistent evidence of increased micronucleus frequency from *medium* confidence studies of the blood, nasal and oral cavity of exposed workers that correlated with blood chromium levels (Long et al., 2019; El Safty et al., 2018; Hu et al., 2018; Sudha et al., 2011) • Ten of 11 *low* confidence studies found increased micronuclei in workers despite differences in population and exposure scenarios (Linqing et al., 2016; Wultsch et al., 2014; Qayyum et al., 2012; Balachandar et al., 2010; Iarmarcovai et al., 2005; Danadevi et al., 2004; Medeiros et al., 2003; Benova et al., 2002; Vaglenov et al., 1999) • Consistent evidence of increased chromosomal aberrations in *low* confidence studies of workers despite sensitivity concerns that biased toward the null (Balachandar et al., 2010; Halasova et al., 2008; Maeng et al., 2004; Deng et al., 1988; Koshi et al., 1984; Sarto et al., 1982) • Increased mutation frequency in the lungs of transgenic rodents exposed via intratracheal instillation, increasing with dose and post-exposure time, provides biological plausibility for mutations in exposed target tissues (Cheng et al. (2000; 1998)) • Consistent supporting evidence of genotoxicity in studies of exposed humans and animals dosed via i.p. injection, including DNA strand breaks, adducts, crosslinks, or other DNA damage and repair-related endpoints (e.g., sister chromatid exchange) (See Appendix C.3.3, Table C-59) • Correlation of systemic Cr levels and other genotoxic endpoints (El Safty et al., 2018; Qayyum et al., 2012; Sudha et al., 2011; Danadevi et al., 2004) • Correlation of MN with work duration (Danadevi et al., 2004) *Oral exposure:* • Some mixed evidence of micronucleus frequency in one *low* confidence study in the bone marrow of Cr(VI)-exposed mice (NTP, 2007) and positive findings of mutation in two *low* confidence studies in the developing mouse fetus (Schiestl et al., 1997) and in male rat germ cells (Marat et al., 2018) • Largely null findings of gene mutation or micronuclei in *low* confidence studies in the bone marrow (De Flora et al., 2006; Mirsalis et al., 1996; Shindo et al., 1989) or GI tract (Aoki et al., 2019; Thompson et al., 2015c; Thompson et al., 2015b; O'Brien et	exposed humans, supported by a small number of low confidence studies in animals exposed via inhalation or ingestion, with other supporting evidence of genotoxicity provided by supplemental studies humans, animals, and in vitro.

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
	al., 2013) of mice or rats, though all but one of these studies lacked sensitivity for detection due to nontoxic dose ranges tested *In vitro:* • DNA reactivity and genotoxicity has been confirmed in a large evidence base of in vitro studies (see Appendix C.3.4)	
Cytotoxicity and degenerative cellular changes (Sections 3.2.1, 3.2.2, 3.2.3.4)	*Biochemical markers of cell injury in the lung:* • Concentration-related increases in total protein, albumin, and LDH activity have been observed in rats exposed via inhalation for 30 and 90 days to ≥0.05 mg/m^3 Cr(VI) (Glaser et al., 1990) *Atrophy and blunting of small intestinal villi:* • Observed to increase with dose in mice following drinking water exposures to ≥11.6 Cr(VI)/kg-d after 7 and 90 days (Thompson et al., 2011) • Observed in a significant proportion of mice at all doses after 90 day (≥3 mg Cr(VI)/kg-d) or 2 year (≥0.3 mg/kg-d) drinking water exposures in mice (not observed in rats) (NTP, 2008, 2007) • Also observed in rats at 7.2 mg Cr(VI)/kg-d in drinking water (Thompson et al., 2012b) *Cytoplasmic vacuolization of small intestinal villi:* • Observed in mice following ≥11.6 mg Cr(VI)/kg-d in drinking water for 7 days and ≥4.6 mg Cr(VI)/kg-d in drinking water for 90 days (not observed in rats) (Thompson et al., 2011) • Observed at all doses (≥3 mg Cr(VI)/kg-d) in drinking water after 90 days exposure in drinking water (qualitative data) (not observed in rats) (NTP, 2007) *Apoptosis in the lung and small intestine:* • Lung: One intratracheal instillation exposure study in rats observed increased apoptosis in bronchial epithelium and lung parenchyma; in vitro studies support dose and time-dependent increases in apoptosis following Cr(VI) exposure in human lung cells (Reynolds et al., 2012; Azad et al., 2008; Reynolds and Zhitkovich, 2007; Gambelunghe et al., 2006; D'Agostini et al., 2002; Carlisle et al., 2000) • Small intestine, mouse: Apoptotic villi increasing with dose ≥11.6 Cr(VI)/kg-d in drinking water for 90 days; not observed after 7 days (Thompson et al., 2015b; Thompson et al., 2015a; O'Brien et al., 2013; Thompson et al., 2011)	Consistent evidence of cytotoxicity and degenerative cellular changes observed in the lung and small intestine of animals following inhalation and drinking water exposures, respectively.

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
	• Small intestine, rat: Apoptotic villi at ≥7.2 mg Cr(VI)/kg-d in drinking water (Thompson et al., 2012b)	
Suppression of apoptosis (Section 3.2.3.4; Appendix C.3.2.10 and C.3.3)	*Oral exposures:* • Inhibition of the MAPK inhibitor RKIP was observed in the stomach and colon of male Wistar rats after 60 days of exposure to Cr(VI) in drinking water, leading to the activation of the ERK/MAPK signaling pathway (Tsao et al., 2011) • Activation of the ERK/MAPK signaling pathway promotes cell proliferation (via c-Myc expression activation) and has been observed in rat stomach and colon after oral exposure (Tsao et al., 2011)	Biologically plausible evidence of the suppression of apoptosis, a hallmark of cancer, in the stomach and colon of animals exposed via drinking water.
Cell proliferation (Section 3.2.3.4; Appendix C.3.2.10 and C.3.3)	*Inhalation exposures:* • Cyclin D1, a regulator and promoter of cell cycle progression, has been detected at significantly increased levels in the lung tumor tissues of chromate-exposed patients compared to unexposed lung cancer patients. Increased expression of cyclin D1 has been associated with cell proliferation and tumorigenesis (Katabami et al., 2000) *Oral exposures:* • The cellular replication marker Ki-67, which is upregulated in human intestinal adenomas, has been found to be increased in isolated duodenal mucosal cells from the small intestine of mice exposed to Cr(VI) via drinking water for 7 and 90 days (Rager et al., 2017; Kopec et al., 2012a) • The c-Myc oncogene codes for a pro-proliferation transcription factor and can be activated by Wnt or the MAPK/ERK pathway, though it can also be blocked by NF-κB signaling. A dose-dependent increase in the c-Myc oncogene was found in the stomach and colon of male Wistar rats after 60 days of exposure to Cr(VI) in drinking water (Tsao et al., 2011) • Galectin-1, associated with gastric cancer cell motility and overexpressed in gastric tumor cells and digestive cancers, was increased in the stomach and colon of male Wistar rats after 60 days of exposure to Cr(VI) in drinking water (Tsao et al., 2011)	Biologically plausible evidence of increased cell proliferation, a hallmark of cancer, as interpreted by the aberrant expression of genes related to cell cycle regulation in lung tumor tissues of humans exposed to Cr(VI) and in the stomach, duodenum and colon of animals exposed via drinking water.
Regenerative hyperplasia (Sections 3.2.1, 3.2.2, 3.2.3.4)	*Focal epithelial hyperplasia of the small intestine:* • Observed in mice exposed to ≥1.18 mg (females) and ≥2.4 mg (males) Cr(VI)/kg-d in drinking water for 2 years. The responses were not statistically significant, but this is	Consistent evidence of hyperplasia interpreted to be the result of regeneration following cell injury following oral exposures in the small intestine of mice and rats and

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
	considered a biologically significant pre-neoplastic lesion due to morphologic similarity to adenoma (NTP, 2008) *Diffuse epithelial hyperplasia of the lung and small intestine:* • Lung: Bronchioalveolar hyperplasia (70–100%) observed in rats following 0.050–0.40 mg/m^3 Cr(VI) inhalation exposure for 30 days, but incidence was decreased at 90 days (Glaser et al., 1990) • Small intestinal crypt cells, mice: Hyperplasia reported in mice exposed for 7 days at 31.1 mg Cr(VI)/kg-d (NS) in drinking water with no changes in mitotic activity in crypt cells and following 90 days at ≥11.6 mg Cr(VI)/kg-d (non-dose-dependent) (Thompson et al., 2015b; Thompson et al., 2011) • Small intestine, mice: Hyperplasia observed at all doses (≥3 mg Cr(VI)/kg-d) in drinking water for 90 days, minimal to mild severity, 100% incidence at mid/high dose levels, with increased numbers of mitotic figures in the hyperplastic epithelium (in females and four male datasets in multiple strains). Also observed at all doses (≥0.3 mg Cr(VI)/kg-d) in drinking water for 2 years, increasing with dose, minimal to mild severity, with increased numbers of mitotic figures in the hyperplastic epithelium (NTP, 2008, 2007) • Small intestinal villous cells, rats: Hyperplasia observed at ≥7.2 mg Cr(VI)/kg-d in drinking water for 7 and 90 days (Thompson et al., 2012b)	following inhalation exposures in the lung in rats.
Inflammation (Section 3.2.3.4; Appendix Table C-38)	*In the lung:* • Increases in macrophages in BALF at 0.9 mg/m^3 Cr(VI) inhalation exposure for 4-6 weeks in rabbit and at at 0.20 and 0.40 mg/m^3 Cr(VI) for 30 and 90 days in rats (Glaser et al., 1990; Johansson et al., 1986b) • In rats exposed for 28 and 90 days, increased lymphocytes in BALF at 0.025 mg/m^3 and 0.05 mg/m^3 Cr(VI); increased granulocytes/neutrophils at 0.05 mg/m^3 Cr(VI); no change or decreased number of macrophages at 0.050 and 0.20 mg/m^3 Cr(VI) inhalation exposure. In rats exposed for 4-48 weeks, increased granulocytes/neutrophils; no change or decreased number of macrophages at 0.36 mg/m^3 Cr(VI) inhalation exposure (Cohen et al., 2003; Glaser et al., 1985) • Histiocytosis (macrophage accumulation) associated with inflammation observed in rats and rabbits exposed via inhalation for 30-90 days (Kim et al., 2004; Glaser et al., 1990; Johansson et al., 1986a)	Consistent evidence of chronic inflammation, an enabling characteristic of cancer, has been observed in animals following Cr(VI) inhalation. There is no histopathological evidence consistent with chronic inflammation reported following oral exposures in animals, although some indirect evidence consistent with inflammation has been reported.

Biological events (and relevant sections)	Summary of key findings	Interpretations, judgments and rationale
	In the GI tract: • Cytokine fluctuations observed in the duodenum (and not the oral mucosa) of mice (↓ IL-1β and TNF-α) and rats (↑ IL-1α, IL-6; ↓ IL-4) following Cr(VI) exposure in drinking water • Induction of proinflammatory signaling pathways (e.g., NF-κB) in animals following oral exposures	

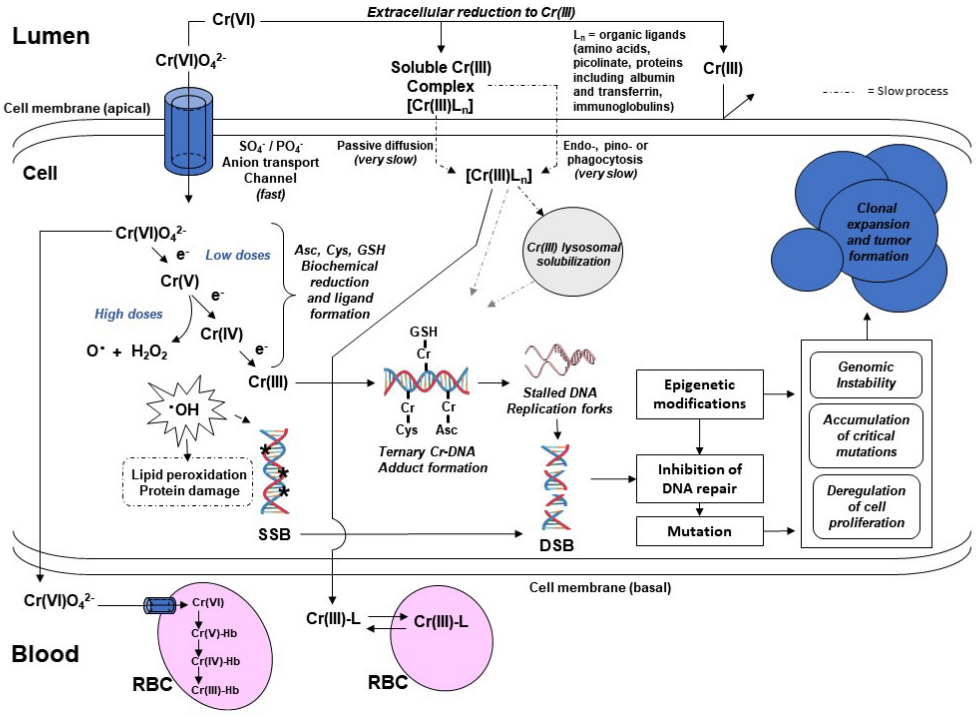

Figure 3-18. Cellular processes involved in the mutagenic MOA of Cr(VI).

Susceptible populations

A number of different factors were identified that could predispose some populations of humans to be more susceptible to Cr(VI) carcinogenicity when ingested.

Low stomach acid

Because extracellular reduction of Cr(VI) to Cr(III) serves as a detoxifying mechanism, conditions that would lower an individual's ability to effectively reduce Cr(VI) could lead to a higher rate of Cr(VI) absorption into the cells lining the GI tract. Following oral ingestion, gastric emptying to the small intestine competes with the rapid extracellular reduction to Cr(III) by gastric juices (Proctor et al., 2012; De Flora et al., 1997). However, there is significant interindividual variability of stomach pH in the human population. Individuals taking medication to treat gastroesophageal reflux disease (GERD), including calcium carbonate-based acid reducers and proton pump inhibitors, have an elevated stomach pH during treatment. Individuals with a preexisting low stomach acid condition (hypochlorhydria, also known as achlorhydria) consistently have a high gastric pH of approximately 8 (Kalantzi et al., 2006; Feldman and Barnett, 1991; Christiansen, 1968). This condition may be caused or exacerbated by multiple other preexisting gastric conditions, including *H. pylori* infection. Gastric pH also varies by age, with neonates notably having neutral stomach pH at birth (Neal-Kluever et al., 2019). The prevalence of hypochlorhydria (see above) is believed to be high in elderly populations (age 65 and up) (Doki et al., 2017). The general healthy population also exhibits high variability in stomach pH. Among adults without

hypochlorhydria and who do not regularly take antacids, 5% of men may exhibit basal pH exceeding 5, and 5% of women may exhibit basal pH exceeding 6.8 (Feldman and Barnett, 1991).

Genetic polymorphisms

Individuals with genetic polymorphisms conveying deficiencies in DNA repair capacity may have increased susceptibility to Cr(VI)-induced lung cancer. Several studies in humans have identified polymorphisms in genes related to DNA repair and tumor suppression that were correlated with increased genetic damage and lung cancer (summarized above and in Appendix C.3.14; see also (Urbano et al., 2012)). DNA adducts formed directly by chromium or indirectly via oxidative damage are substrates for nucleotide excision repair (for bulky lesions) and mismatch repair (for misincorporated bases during DNA replication and homologous recombination); heritable deficiencies in the effectiveness of these repair processes can cause a higher rate of unprocessed genetic damage leading to the formation of heritable mutations.

Carriers of the cystic fibrosis mutant allele

The analyses by US EPA (see Appendix C.3.4.2 and Mezencev and Auerbach (2021)) of the toxicogenomic data reported in Kopec et al. (2012b; 2012a) from mice exposed to Cr(VI) (reviewed earlier in this section) have identified a potential role for CFTR in the carcinogenic effects of Cr(VI). Tumorigenicity of impaired CFTR activity in animal models supports the relevance of the Cr(VI)-mediated inactivation of CFTR for the development of small intestinal tumors in mice exposed to Cr(VI) in drinking water (Than et al., 2016), and CFTR has been shown to act as a tumor suppressor in the human colon (Than et al., 2016). These findings may indicate that carriers of the mutated CFTR allele could be more sensitive to the Cr(VI)-mediated carcinogenicity. In the US alone, more than 10 million people are carriers of a mutated CFTR allele that confers an approximate 50% reduction in CFTR expression levels. Although these individuals do not develop cystic fibrosis, the deficit in CFTR function has been shown to lead to an increased risk for several conditions associated with the disease, including colorectal cancer (OR = 1.44, 95% CI: 1.01–2.05) (Miller et al., 2020). CFTR suppression induced by low Cr(VI) exposures in drinking water can be expected to occur in all exposed populations, but a more significant effect would be expected in humans already producing low levels of this protein.

3.2.3.5. *Integration of Evidence for Cancer of the GI Tract*

The integrated evidence for Cr(VI)-induced cancer of the GI tract is summarized in Table 3-22. Overall, Cr(VI) is **likely to be carcinogenic** to the human GI tract by the oral route of exposure. This conclusion is based on *robust* evidence of cancer from a *high* confidence 2-year cancer bioassay conducted by NTP, which showed a statistically significant increase in oral cavity tumors in male and female F344/N rats and small intestine neoplasms in B6C3F1 male and female mice (NTP, 2008). Notably, at the lower doses where tumor occurrence was nonsignificant compared to concurrent controls, incidences exceeded NTP historical controls in both species. Therefore, some

tumors that were not statistically significant may be biologically significant due to the increasing trend and low historical control incidence (Appendix D.5).

The evidence of carcinogenicity of the GI tract from human studies is *slight*. Results for two populations exposed to Cr(VI) through drinking water in China and Greece were available in the epidemiological evidence base that analyzed stomach cancer risk (Linos et al., 2011; Kerger et al., 2009; Beaumont et al., 2008). The studies reported increased SMRs when their mortality experience was compared to other communities in the surrounding areas or to the mortality experience in the province where the exposed communities were located. While uncertainties in the study methods and analyses resulted in low confidence ratings, the studies in both populations reported increased risk estimates supporting a judgment of *slight*.

The summary effect estimates from the meta-analysis of GI tract cancer risk from the occupational studies of workers with inhalation exposure to Cr(VI) showed small increases in risk for each cancer site, and this increase was statistically significant for rectal cancer. There were few studies reporting odds ratios, but in each case (esophagus and stomach), summary effect estimates based on these studies were somewhat higher compared with summary estimates based on other relative risk measures (although neither odds ratio-based estimate was statistically significant). However, there were not clear patterns of risk by either occupational group or specific cancer site. There is more coherence for the results for colon cancer when stratified by occupational grouping (Appendix Table C-45; Appendix Figure C-19) since the occupations with a higher certainty of exposure to Cr(VI) showed higher summary effect estimates, although inconsistencies remain among the studies overall.

Looking across the available evidence in animals and humans, while interspecies correlation is lacking for the exact tumor site within the intestinal tract, the overall species concordance, spanning the entire alimentary tract, including the oral cavity, is robust. Although it is difficult to draw conclusions regarding an association between human exposure to Cr(VI) through drinking water and GI tract cancer from the available epidemiological evidence, there is consistency among species (human, rat, and mouse) regarding the potential for Cr(VI) to cause cancer at various sites along the GI tract.

Potential MOAs for carcinogenicity induced by ingested Cr(VI) in the mouse small intestine include mutagenicity and regenerative proliferation caused by tissue injury leading to a higher probability of the clonal outgrowth of spontaneous mutations. These mechanistic processes are not mutually exclusive, and there is evidence that Cr(VI)-induced carcinogenesis in the GI tract after oral exposure involves both MOAs.

Bioavailability results and kinetic considerations (see Section 3.1 and Appendix C.1) lead to the evidence-based interpretation that approximately 10–20% of ingested low dose Cr(VI) escapes human gastric inactivation and could therefore reach the target cells in appreciable amounts and would thus be reasonably anticipated to act as a mutagen in the GI tract epithelium. Given the cellular capacity for uptake of Cr(VI) in highly absorptive intestinal tissues, it is biologically

plausible that Cr(VI) can induce genetic damage in the human GI tract. By assuming significant (80–90%) but incomplete gastric detoxification, the capacity for autonomous growth may remain latent for weeks, months, or years, during which time an initiated cell may be phenotypically indistinguishable from other parenchymal cells in that tissue. The average tumor diagnosis was over 700 days (100 weeks) for both sexes of mice (first onset at 451 days and most observed at terminal sacrifice). Most human and animal neoplasms studied to date are of monoclonal origin. There are several salient characteristics of initiation. It can occur following a single exposure to a known carcinogen. Changes produced by the initiator may be latent for weeks or months and are considered irreversible. The hyperplasia observed at the 2-year evaluation endpoint may, therefore, be a manifestation of intestinal responses to late clonal expansion following an early initiation. Also, with age, spontaneous DNA replication becomes more error prone resulting in small intestinal tumors. Therefore, the hyperplastic changes described could support either MOA (cytotoxicity with regenerative cell proliferation and mutagenicity).

The hypothesis that continuous wounding results in regenerative proliferation that may give rise to spontaneous mutations progressing to neoplasia is largely supported by histopathological findings that indicate degenerative changes including villous blunting/atrophy accompanied by cytoplasmic vacuolization and crypt hyperplasia. Importantly, it is unlikely that this MOA is solely operational in the intestinal tumors observed by NTP after 2 years. While a 'wounding and regenerative cell proliferation' MOA is supported by short-term (7 and 28 day) and subchronic (90 day) bioassays, these studies were (a) too short in duration to show that regenerative hyperplasia progressed to tumor formation (resulting in a threshold dose) and (b) did not demonstrate that a mutagenic MOA could reliably be excluded. Therefore, whether the clonal selection and outgrowth of spontaneous mutations is responsible for Cr(VI) tumorigenesis remains a data gap; DNA sequencing data may assist with assessing the validity of this hypothesis.

No direct mechanistic evidence in the rat oral mucosa is available to support an MOA for tumorigenesis of the rat oral cavity induced by ingested Cr(VI). It is important to note that the apical membrane of the human tongue, oral mucosa, and esophagus will come into direct contact with Cr(VI) in ingested drinking water before gastric detoxification. This is supported by consistent observations of increased micronuclei in oral epithelial cells from humans occupationally exposed to Cr(VI). Importantly, the proposed wounding and regenerative proliferation MOA for the intestinal tumors in mice does not address the Cr(VI) oral cavity tumors of rats, in which neither degenerative changes nor hyperplasia were observed. Only one *low* confidence study investigated the mutation frequency in the rat oral cavity and did not find an increase after a 7-day exposure to Cr(VI) in drinking water. Additional studies designed to be sensitive for detecting mutations as well as other potential mechanisms involved in carcinogenicity of the oral mucosa are needed.

Overall, the determination of a mutagenic MOA, the incompleteness of gastric detoxification, and the development of oral cavity tumors without any apparent tissue injury or regenerative

proliferation argue against a threshold for low dose extrapolation of cancer risk for both oral and GI tract tumors from ingested Cr(VI).

Table 3-22. Evidence profile table for cancer of the GI tract[a]

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from studies of exposed humans					⊕⊕⊙
GASTRIC CANCER (ORAL) **Low confidence:** Beaumont et al. (2008) Kerger et al. (2009) Linos et al. (2011)	Results for two populations in China and Greece exposed to Cr(VI) in drinking water showed increased SMRs.	• Consistent observations of elevated risk using two comparisons (i.e., surrounding regions and entire province) in separate geographical locations	• Ecological study designs (lack of individual estimates of exposure) • Uncertain nature of the mortality data for that period in China • Potential impact of confounding by differences in SES between comparison groups • Imprecise estimates changed in magnitude depending on how referents were defined	⊕⊙⊙ *Slight* Although increased SMRs were found in two studies of two populations exposed to Cr(VI) in drinking water when comparing mortality to unexposed communities in the surrounding areas, these estimates were imprecise and changed in magnitude depending on the definition of the unexposed communities. While uncertainties in the study methods and analyses resulted in low confidence ratings, the studies in both populations reported increased risk estimates supporting a judgment of slight.	**Cr(VI) is likely to be carcinogenic to humans** via the oral route of exposure. Robust evidence shows tumors of the GI tract in mice (small intestine) and rats (oral cavity) in both sexes; the oral cavity tumors were rare indicating increased biological significance. Evidence from humans is slight but is consistent in reporting some risk of cancers of the GI tract in humans exposed via drinking water or mucociliary clearance. Biological plausibility for the small intestinal tumors is provided by histopathological evidence of tissue degeneration and

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
GI TRACT CANCER (INHALATION/ORAL) Medium confidence: 43 occupational studies of cancer mortality or incidence	A meta-analysis of GI tract cancer risk from occupational studies of workers with inhalation and oral (swallowing dust) exposure to Cr(VI) showed small increases in risk for each cancer site. The summary estimates for SMR/SIR analyses of rectal cancer were statistically significant. The summary estimates for the few studies reporting odds ratios (esophagus and stomach) were somewhat higher (although neither odds ratio-based estimate was statistically significant).	• Large number of studies contributed SMR/SIR results for four cancer sites (esophagus, stomach, colon and rectum) • Occupations with a higher certainty of exposure to Cr(VI) showed higher summary effect estimates	• There were no coherent patterns of risk when looking at cancer sites across occupational groupings or at occupational groups across cancer sites • Variation in the prevalence, frequency and magnitude of exposure is likely within the exposure groups, which would result in an underestimate of the estimated risk	⊕⊙⊙ *Slight* Although the risk estimate for rectal cancer was statistically significant, and coherent results for colon cancer risk were found when stratified by occupational groupings expected to have higher exposures to Cr(VI), inconsistencies in patterns of risk across occupational groups raise uncertainties.	hyperplasia in the small intestine of mice and molecular evidence of cell proliferation and oxidative stress in these animals prior to tumor formation. A primary role for mutagenicity, evident in oral cavity tissues of exposed humans and known to occur when Cr(VI) comes into direct contact with cells, in GI tract tumorigenesis (and in particular, in tumors of the rat oral cavity) is not clear but also cannot be ruled out. Susceptibility is assumed for humans with impaired ability to reduce Cr(VI) in the stomach.

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from animal studies					
GI TRACT TUMORS (ORAL) **High confidence:** NTP (2008)	Statistically significant increases in tumors of the GI tract were reported in a high confidence 2-year animal bioassay: adenomas and carcinomas of the small intestine (male and female mice), and squamous cell carcinomas and papillomas of the oral mucosa and tongue (male and female rats). Tumors of the oral cavity and small intestine have a very low historical incidence.	• Consistent findings in one high confidence 2-year study that contained bioassays in rats and mice of both sexes • Coherent, biologically related findings within the GI tract • Large magnitude of effects • Strong dose-response gradient • Mechanistic evidence provides biological plausibility	• None	⊕⊕⊕ *Robust* Consistent findings in one large *high* confidence study finding tumors in the GI tract in two species and both sexes Animal mechanistic evidence informing biological plausibility (hyperplasia in mouse small intestine may be a precursor event for tumors)	
HISTOPATHOLOGICAL CHANGES (ORAL) **High confidence:** NTP (2008) NTP (2007) Thompson et al. (2012b) Thompson et al. (2011)	All studies examining effects in the small intestine are rated high confidence. Degenerative changes in intestinal villi and hyperplasia of the small intestine observed in male and female mice by NTP (2008, 2007), and in female	• Consistent findings in four high confidence chronic and subchronic studies that contained multiple bioassays in rats and mice of both sexes, and	• Inconsistent observations of hyperplasia between mice and rats, though this is explained in part by pharmacokinetic differences	⊕⊕⊕ *Robust* Histopathological changes reported in high confidence studies (tissue injury and proliferative changes) observed across the animal evidence base	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	mice and rats by Thompson et al. (2012b; 2011). Histiocytic cellular infiltration observed in the small intestine of male and female rats and mice in all studies and bioassays.	multiple strains of mice • Large magnitude of effects • Strong dose-response gradient • Mechanistic evidence provides plausibility • Coherence as potential preneoplastic lesions in the mouse small intestine only		database are coherent following chronic and/or subchronic oral exposures in rats and mice and suggest adverse effects of Cr(VI) on the GI tract, findings that are supported by mechanistic evidence. Inconsistency between species (mice consistently exhibited hyperplasia of the small intestine, whereas results in rats were mixed) is partly explained by pharmacokinetics	

[a]See Table 3-21 for the summary of key mechanistic events involved in Cr(VI)-induced cancer.

3.2.4. Hepatic effects

The liver is a common site of toxicity as it functions to metabolize exogenous as well as endogenous chemicals. The liver is considered an accessory digestive organ because it synthesizes proteins and compounds necessary for digestion as well as filtering and metabolizing nutrients and toxicants absorbed by the small intestine (first-pass effect). The liver also metabolizes chemicals absorbed into the bloodstream from other routes (such as intravenous injection or inhalation). Because of the first-pass effect, the liver may be affected more severely by toxic chemical exposure via the oral route as compared to the inhalation route.

3.2.4.1. *Human Evidence*

Study evaluation summary

There are four studies that reported on the association between Cr(VI) exposure and hepatic-related clinical chemistries, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP). Increases in serum ALT and AST are considered indicative of hepatocellular damage, with ALT considered to be the more sensitive and specific indicator (EMEA, 2008; Boone et al., 2005). Increases in ALP can be associated with liver cholestasis, however, ALP is not as specific to liver injury as extrahepatic sources of ALP exist (Boone et al., 2005). Other serum measures evaluated, which can help inform liver toxicity, included bilirubin, albumin, total protein, creatinine, and albumin/globulin ratio. In general, increased serum bilirubin and decreased serum albumin/total protein can indicate impaired liver function (EMEA, 2008; Boone et al., 2005).

With respect to confidence in the human studies, one study (Khan et al., 2013) was classified as *uninformative* because exposure was based on tannery work, and there was insufficient information provided on the specific tanning processes used at the facility[32]. This study was not considered further. The three remaining studies were included and classified as *low* confidence (see Table 3-23), with two (Saraswathy and Usharani, 2007; Lin et al., 1994) in occupational populations with exposure primarily via inhalation and one (Sazakli et al., 2014) in the general population with exposure primarily via the oral route. Lin et al. (1994) had adequate exposure measurement due to use of air sampling with appropriate methods and categorization into three levels of exposure. In the remaining two studies, the primary limitation was *deficient* exposure measurement, primarily due to concerns about potential for nondifferential exposure misclassification that would be likely to bias the results towards the null (Sazakli et al., 2014; Saraswathy and Usharani, 2007). In Sazakli et al. (2014), exposure was estimated based on water intake and blood and hair Cr concentrations,

[32]Leather tanning processes that can potentially lead to Cr(VI) exposure include: (1) use of a two-bath process, (2) on-site production of tanning liquors, and (3) leather finishing steps that involve Cr(VI) (e.g., use of Cr(VI)-containing pigments) (Shaw Environmental, 2006). If these processes are not specified by the study, it cannot be determined whether exposure was to Cr(VI) or Cr(III).

but there were poor correlations across measures. In Saraswathy and Usharani (2007), no air data was available and there was no quantitative measurement of exposure. These considerations on exposure measurement are the primary basis for the clinical chemistry outcome judgments presented in Table 3-23.

Table 3-23. Summary of human studies for Cr(VI) hepatic effects and overall confidence classification [high (H), medium (M), low (L)] by outcome. Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Exposure Measurement	Study Design	Clinical Chemistry
Lin et al. (1994)	Chrome plating	Taiwan	Urine, Air, Work category	Cross-sectional	L
Sazakli et al. (2014)	General population	Greece	Urine, Hair, Modeled lifetime Cr(VI) exposure dose	Cross-sectional	L
Saraswathy and Usharani (2007)	Chrome plating	India	Work category	Cross-sectional	L
Khan et al. (2013)	Tannery	Pakistan	Blood, Urine, Work category	Cross-sectional	U

Synthesis of evidence in humans

Two studies (Sazakli et al., 2014; Saraswathy and Usharani, 2007) reported statistically significant changes consistent with liver dysfunction in at least one of the tests (i.e., higher levels of ALT, AST, ALP, or bilirubin and/or lower levels of total protein or albumin with higher exposure) as shown in Table 3-24. These associations were observed despite the potential for exposure misclassification that may have reduced sensitivity. Saraswathy and Usharani (2007) observed an exposure-response gradient across the three exposure categories for ALT, AST, ALP, and total protein. However, there is some inconsistency in the direction of results for total protein and albumin between the two studies. The third study (Lin et al., 1994) evaluated serum ALT, AST, creatinine, and albumin/globulin ratio. The study authors did not report quantitative results but reported that there were no significant differences among workers in the four exposure groups.

Table 3-24. Associations between Cr(VI) and liver clinical chemistries in epidemiology studies

Reference, confidence	Population	Exposure comparison and effect estimate	ALT	AST	ALP	Total protein	Other
Sazakli et al. (2014), low confidence	Cross-sectional in Greece, general population; two drinking water exposure groups (n = 237) and controls (n = 67)	Regression coefficients for calculated lifetime exposure dose and hair biomarkers	Lifetime: −0.03 (for ln-ALT) Hair: 0.05 (for ln-ALT)	Lifetime: 0.04 Hair: 0.04	Lifetime: 0.12* Hair: 0.22*	Lifetime: 0.14* Hair: 0.24*	Lifetime: Albumin 0.21* Bilirubin −0.11 Hair: Albumin 0.23* Bilirubin −0.07
Saraswathy and Usharani (2007), low confidence	Cross-sectional in India, two chrome plater groups (n = 130) and male area residents (n = 130)	Means ± SD for control/ exposed 8–15 yrs (A)/ exposed 16–25 yrs (B)	Control: 22.0 ± 1.7 Exposed A: 34.3 ± 2.5* Exposed B: 43.3 ± 1.7*	Control: 19.2 ± 2.1 Exposed A: 32.9 ± 3.7* Exposed B: 38.6 ± 4.0*	Control: 60.8 ± 5.7 Exposed A: 70.2 ± 6.2* Exposed B: 83.7 ± 7.6*	Control: 7.8 ± 0.4 Exposed A: 7.5 ± 0.1* Exposed B: 6.1 ± 0.1*	NR
Lin et al. (1994), low confidence	Cross-sectional in Taiwan, three chrome plater groups (n = 79) and aluminum plater referent group (n = 40)	Analysis and quantitative results not reported.	ALT, AST, serum creatinine and albumin/globulin ratio evaluated, however, authors report no significant difference among workers across exposure groups (results not shown).				

*$p < 0.05$.
NR: not reported

In addition, four studies (presented in five publications) reported on mortality attributable to cirrhosis of the liver, all based on occupational cohorts (Birk et al., 2006; Moulin et al., 2000; Moulin et al., 1993b; Moulin et al., 1993a; Moulin et al., 1990). These studies indicated no increase in cirrhosis mortality with higher exposure levels, but this evidence is considered inadequate to assess the association with Cr(VI) due to several limitations, including lack of control of potential confounding (such as by alcohol consumption), concerns about sensitivity and specificity of the exposure measures, and the sensitivity of mortality as the outcome measure.

Overall, there is an indication in the available human studies that higher Cr(VI) exposure may be associated with increased liver dysfunction, but there is some inconsistency in the available results and study evaluation concerns, especially with respect to exposure measurement.

3.2.4.2. *Animal Evidence*

Study evaluation summary

Information relevant to the evaluation of an association between Cr(VI) exposure and liver effects comes from oral and inhalation studies in mice and rats involving subchronic, chronic, and gestational exposures. Liver effects evaluated in this synthesis include changes in liver histology, clinical chemistry, and relative liver weight. As displayed in Table 3-25, studies reporting liver effects in the Cr(VI) evidence base were of varying study quality (based on factors including strength of study design and transparency of reporting), with the most informative evidence from the NTP chronic and subchronic drinking water bioassays in rats and mice (NTP, 2008, 2007).

Table 3-25. Summary of included animal studies for Cr(VI) liver effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive data graphic for rating rationales.

				Liver outcomes		
Author (year)	**Species (strain)**	**Exposure design**	**Exposure route**	**Organ weight**	**Clinical chemistry**	**Histopathology**
NTP (1997)	Mouse (BALB/c)	Reproductive study-continuous breeding (F0 to F2)	Diet	H		H
NTP (1996a)	Mouse (BALB/c)	Subchronic	Diet			H
NTP (1996b)	Rat (Sprague-Dawley)	Subchronic	Diet			H
NTP (2007)	Rat (F344/N); Mouse (B6C3F1, BALB/c, C57BL/6)	Subchronic	Drinking water	H	H	H
NTP (2008)	Rats (F344/N); Mouse (B6C3F1)	Chronic	Drinking water		H	H
Wang et al. (2015)	Rat (Sprague-Dawley), male	Subchronic	Drinking water	M	M	M
Elshazly et al. (2016)	Rat (Sprague-Dawley)	Subchronic	Drinking water	-	M	M
Chopra et al. (1996)	Rat (Wistar), female	Subchronic	Drinking water	M	L	L
Rafael et al. (2007)	Rat (Wistar)	Subchronic	Drinking water	-	M	L
Acharya et al. (2001)	Rat (Wistar), male	Chronic	Drinking water		L	L
Krim et al. (2013)	Rat (Albino)	Subchronic	Gavage	-	M	-
Navya et al. (2017a)	Rat (Wistar), male	Subchronic	Gavage	-	M	L
Mo et al. (2018)	Rabbit (New Zealand), male and female	Subchronic	Gavage	-	-	L

				Liver outcomes		
Author (year)	**Species (strain)**	**Exposure design**	**Exposure route**	**Organ weight**	**Clinical chemistry**	**Histopathology**
Meenakshi et al. (1989)	Rat (Wistar)	Subchronic	Gavage	-	L	U
Kim et al. (2004)	Rat (Sprague-Dawley)	Subchronic	Inhalation	M	M	-
Glaser et al. (1985)	Rat (Wistar)	Subchronic	Inhalation	L	L	M
Glaser et al. (1986)	Rat (Wistar)	Chronic	Inhalation	L	L	U

[a]Five studies reporting liver endpoints met PECO criteria but were considered to be *uninformative* at the study evaluation stage: (Sánchez-Martín et al., 2015; Soudani et al., 2013; Geetha et al., 2003; Kumar and Barthwal, 1991; Nettesheim et al., 1971).

Synthesis of evidence in animals

Histopathology

Several subchronic and chronic studies in rats and mice reported histological lesions in the liver associated with oral exposure to Cr(VI). These lesions include increased inflammation and infiltration of immune cells (Elshazly et al., 2016; NTP, 2008, 2007), cytoplasmic vacuolation (fatty changes) (Elshazly et al., 2016; NTP, 2008; Acharya et al., 2001; NTP, 1997; Chopra et al., 1996; NTP, 1996a), indications of apoptosis and necrosis (Elshazly et al., 2016; Acharya et al., 2001; Chopra et al., 1996), and increased hepatocellular foci (Elshazly et al., 2016; NTP, 2008). These findings are presented in more detail below (see also Figure 3-19). While some NTP studies observed histological lesions, several other NTP studies failed to find altered histological findings in the liver. These studies include an oral study that exposed male and female SD rats to doses of up to approximately 10 mg Cr(VI)/kg-day for 9 weeks (NTP, 1996b), as well as a 3-month study in three different strains of mice (NTP, 2007). A 3-month study in B6C3F1 mice reported a lack of histological changes in the liver (NTP, 2007) as well as a 9-week oral study in BALB/c mice (although some non-statistically significant increases in vacuolation were observed) (NTP, 1996a). In addition, no treatment-related lesions in the liver were found in male and female BALB/c F0 or F1 mice exposed orally in a continuous breeding study at doses of 30–50 mg Cr(VI)/kg-day for approximately 20 weeks (NTP, 1997). Across the evidence base, there is some indication that mice may be more resistant than rats to Cr(VI)-induced changes in the liver, and that histological changes that were not observed following subchronic exposure durations may be apparent after chronic exposure. For instance, a study of male and female B6C3F1 mice exposed at doses up to ~28 mg Cr(VI)/kg-day for 12 weeks (NTP, 2007) did not find evidence of liver histological changes; however, after 2 years of exposure, histiocytic infiltration was noted in female mice (but not males) (NTP, 2008).

The available inhalation studies (*medium* and *low* confidence) investigated, but did not observe, histological alterations in the liver in rats exposed for 12 weeks at concentrations of up to 1.25 mg Cr(VI)/m^3 (Kim et al., 2004) or 0.2 mg Cr(VI)/m^3 (Glaser et al., 1985), or for longer durations (18 months followed by a 12 month unexposed period) at concentrations of up to 0.1 mg Cr(VI)/m^3 (Glaser et al., 1986). However, liver chromium concentration following inhalation exposure to Cr(VI) is expected to be approximately 1–2 orders of magnitude lower than concentrations following oral exposure due to the first-pass effect (O'Flaherty and Radike, 1991). As a result, the extent of hepatotoxicity would be expected to differ by route of exposure.

Inflammation-Related Hepatotoxicity

Inflammation-related histological changes in the liver (increased inflammation and infiltration of immune cells) were reported in several *high* confidence studies of Cr(VI) exposure in F344 rats (NTP, 2008, 2007) and B6C3F1 mice (NTP, 2008). In female F344 rats, statistically significantly increased incidences of chronic focal inflammation were reported for females in the highest dose group following 3 months of exposure at 20.9 mg Cr(VI)/kg-day (NTP, 2007) and at lower doses (0.2–7 mg Cr(VI)/kg-day) after two years of exposure, with incidences increasing monotonically with dose (NTP, 2008). In male F344 rats exposed for 3 months, no statistically significant increase in liver lesions was found (NTP, 2007); however, after 2 years of exposure, chronic inflammation was increased in males in the second highest dose group (56%) relative to controls, although control incidence was high (38%) and no clear dose-response was apparent for this endpoint (NTP, 2008). In a 2-year study, a statistically significantly increased incidence of chronic inflammation was observed in female B6C3F1 mice in the second highest exposure group (3.2 mg Cr(VI)/kg-day) but not in other exposed groups (high dose: 8.9 mg Cr(VI)/kg-day) or in male mice at doses up to 5.7 mg Cr(VI)/kg-day (NTP, 2008). Increased Kupffer cell (stellate macrophage) activation was observed in a high dose, *medium* confidence study in male SD rats exposed to approximately[33] 25 mg Cr(VI)/kg-day for six months (Elshazly et al., 2016). In a continuous breeding study in BALB/c mice, no increased inflammatory changes in the liver were observed in F0 or F1 male or female mice exposed for approximately 20 weeks at doses up to 30–50 mg/kg-day (NTP, 1997).

In damaged tissues, infiltrating histiocytes (macrophages) display functions such as modulation of inflammatory cells, removal of damaged tissues/cellular debris, and antigen presentation, as well as fibrogenic stimulation (Yamate et al., 2016). The incidence of infiltration of histiocytes in the liver was statistically significantly elevated in female F344 rats exposed for 3 months at doses ≥3.5 mg Cr(VI)/kg-day (NTP, 2007) and in female F344 rats exposed at lower doses (≥0.96 mg Cr(VI)/kg-day) for two years (NTP, 2008). Histiocytic infiltration was not observed in male F344 rats exposed for 3 months at doses up to 20.9 mg Cr(VI)/kg-day but was

[33] Elshazly et al. (2016) did not contain enough information to accurately calculate a dose in mg/kg-d. Using drinking water factors for SD rats from U.S. EPA (1988), the dose may be as high as 25 mg/kg-d (although this does not take into account decreased palatability of the drinking water at 180 mg/L).

statistically significantly elevated in high dose male rats (5.9 mg Cr(VI)/kg-day) following 2 years of exposure. Increased incidences of minimal to mild histiocytic infiltration were also observed in all exposed groups of female mice (0.3 to 8.9 mg Cr(VI)/kg-day), showing an increasing response with dose, in a 2-year study, but not in male mice (NTP, 2008). Hepatic infiltration of inflammatory cells was also noted in a *medium* confidence study which exposed male rats to approximately 25 mg Cr(VI)/kg-day for six months (Elshazly et al., 2016). NTP (2008) stated that the significance of histiocytic infiltration is unknown but hypothesized that infiltration of macrophages may reflect phagocytosis of an insoluble precipitate. However, it is important to acknowledge that activated macrophages can also damage tissue by secreting cytotoxic factors indicative of an innate inflammatory response and creating an inflammatory environment (Francke and Mog, 2021; Koyama and Brenner, 2017; Yamate et al., 2016) and chronic hepatic inflammation can lead to fibrosis (Koyama and Brenner, 2017). Histiocytic cellular infiltration with exposure to Cr(VI) was also observed in several other tissues (including the duodenum and mesenteric and pancreatic lymph nodes) in both rats and mice (NTP, 2008). See the immune effects section (Section 3.2.6) for further discussion of this effect.

Necrosis and Apoptosis

Few chronic or subchronic studies across the evidence base reported liver necrosis or indications of apoptosis. The incidence of necrosis was not increased in Cr(VI)-exposed animals in the large (50/sex/group), *high* confidence, 2-year NTP bioassay in F344 rats or B6C3F1 mice at doses of up to 6–9 mg Cr(VI)/kg-day (NTP, 2008) or in an NTP continuous breeding study in F0 and F1 BALB/c mice (NTP, 1997). However, a high dose, *medium* confidence study observed necrosis in all SD rats exposed to 25 mg Cr(VI)/kg-day for six months (Elshazly et al., 2016). In addition, several *low* confidence studies (discussed below) of shorter duration in Wistar rats reported evidence of necrosis or apoptosis associated with Cr(VI) exposure. Rafael et al. (2007) described histological changes indicative of apoptosis as well as necrosis in Wistar rats exposed to approximately 3 mg Cr(VI)/kg-day for 10 weeks. This study also reported immunohistochemical evidence for increased expression of caspase-3, a marker for apoptosis, in male rats (Rafael et al., 2007). Mechanistic markers of apoptosis also have been observed with Cr(VI). A 28-day study in male rats gavaged with 10.6 mg Cr(VI)/kg-d reported increased expression of genes involved in apoptosis concurrent with increases in liver enzymes (ALT, AST, and ALP) (Navya et al., 2017a). Regarding evidence of necrosis, two related publications qualitatively described periportal necrosis in Wistar rats exposed to 1.4 mg Cr(VI)/kg-day for 22 weeks (Acharya et al., 2001; Chopra et al., 1996). While low levels of hepatocellular apoptosis may be difficult to detect in chronic and subchronic toxicity studies, numerous short-term mechanistic studies indicate the upregulation of apoptotic genes as well as the detection of specific markers of apoptosis (e.g., caspase-3) following Cr(VI) exposure (see *Mechanistic Evidence* below and Table 3-26).

Fatty Changes and Vacuolation

Fatty changes, or steatosis, the accumulation and retention of fat in hepatocytes, is an early pathological change associated with liver disease. Histologically, fatty change is sometimes noted as vacuolation, with lipid accumulating in hepatocytes as vacuoles. Fatty changes often coincide with hepatic inflammation (Kaiser et al., 2012; Day and James, 1998). If the insult responsible for steatosis persists, more severe pathologies can develop including fibrosis and cirrhosis (Kaiser et al., 2012; Day and James, 1998). Liver vacuolation associated with oral exposure to Cr(VI) was reported in several publications (Elshazly et al., 2016; NTP, 2008; Acharya et al., 2001; Chopra et al., 1996; NTP, 1996a) but not others (NTP, 2007, 1997, 1996b). An increased incidence of scattered hepatocytes with cytoplasmic vacuoles containing lipid, characterized as "fatty changes," was noted in female (but not male) F344 rats at doses ≥ 0.96 mg Cr(VI)/kg-day in the *high* confidence 2-year NTP (2008) study. Furthermore, two similarly designed *low* confidence studies qualitatively reported liver vacuolation in Wistar rats exposed to 1.4 mg Cr(VI)/kg-day for 22 weeks (Acharya et al., 2001; Chopra et al., 1996). A high dose, *medium* confidence study observed vacuolation in all male SD rats exposed to approximately 25 mg Cr(VI)/kg-day for six months (Elshazly et al., 2016). Hepatic vacuolation was also observed in a high confidence study of male and female BALB/c mice exposed via diet at doses ≥5.6 mg Cr(VI)/kg-day for 9 weeks (NTP, 1996a). Study authors reported that the vacuoles were suggestive of lipid accumulation (NTP, 1996a). However, these findings were not supported by other high confidence studies of this strain of mice treated for 3 months (NTP, 2007, 1997) or a similarly designed 9-week study in rats (NTP, 1996b). No increase in the incidence of vacuolation was found at doses up to 8.7 mg Cr(VI)/kg-day in male BALB/c mice in a 3-month study (NTP, 2007) or in F0 male and female BALB/c mice in a continuous breeding study at doses up to ~30–50 mg Cr(VI)/kg-day for approximately 20 weeks (NTP, 1997).

Other Histological Effects

Hepatocellular degeneration, necrosis, altered hepatocellular foci of mixed type, bile duct hyperplasia, oval cell hyperplasia, and periductal fibroplasia were observed in a *medium* confidence study in male SD rats exposed to approximately 25 mg Cr(VI)/kg-day for six months (Elshazly et al., 2016). Other isolated histological changes were reported in the evidence base, including the observation of basophilic hepatocellular foci, a preneoplastic lesion. In F344 rats, authors reported an exposure-related increased incidence of basophilic hepatocellular foci in the 2-year study in male rats, but not in females (NTP, 2008).

Summary of Histological Effects

Overall, there is consistent evidence of Cr(VI)-induced hepatic histological effects, across species and sexes, in animals exposed via the oral route. Increases in chronic inflammation and histiocyte infiltration as well as increased fatty change and associated vacuolation were reported in several *high* confidence studies following chronic and/or subchronic oral exposures in rats and mice. Evidence of cell death (necrosis and apoptosis) was reported in several *low* confidence studies and is supported by short-term mechanistic studies (see Figure 3-19); however, these

endpoints were unchanged in higher confidence studies testing similar doses, for longer durations. Histopathological effects were not observed in *low* and *medium* confidence studies following inhalation exposures, potentially due to differences in target tissue dose across routes of exposure.

In general, female rodents appear to be more sensitive to Cr(VI) induced histological changes (e.g., hepatic inflammation and fatty changes; NTP (2008)). However, few studies are available in the database that evaluated both males and females; most study designs used either male or female animals. In the 2 year rat study (NTP, 2008), chronic inflammation and histiocytic inflammation and were significantly increased in females at lower doses than males (approximately 6–10 fold lower than in male animals).[34] Increased fatty changes were also seen in female rats at doses as low as 0.94 mg/kg-day and were not significantly elevated in males at doses as high as 5.9 mg/kg-d. However, basophilic foci (often considered a preneoplastic effect), was noted in male rats at doses as low as 0.77 mg/kg-d and was not observed in female rats, although male rats were observed to have a much higher background rates of this lesion. For mice, which generally appeared to be less sensitive than rats to hepatic effects with Cr(VI) exposure, statistically significant increases in chronic inflammation and histiocytic infiltration were seen in female, but not male mice (NTP, 2008).[35]

[34]Inflammation: click to see rat data in females and males in HAWC.
Infiltration: click to see rat data in females and males in HAWC.
[35]Inflammation: click to see mouse data in females and males in HAWC. Infiltration: click to see mouse data in females and males in HAWC.

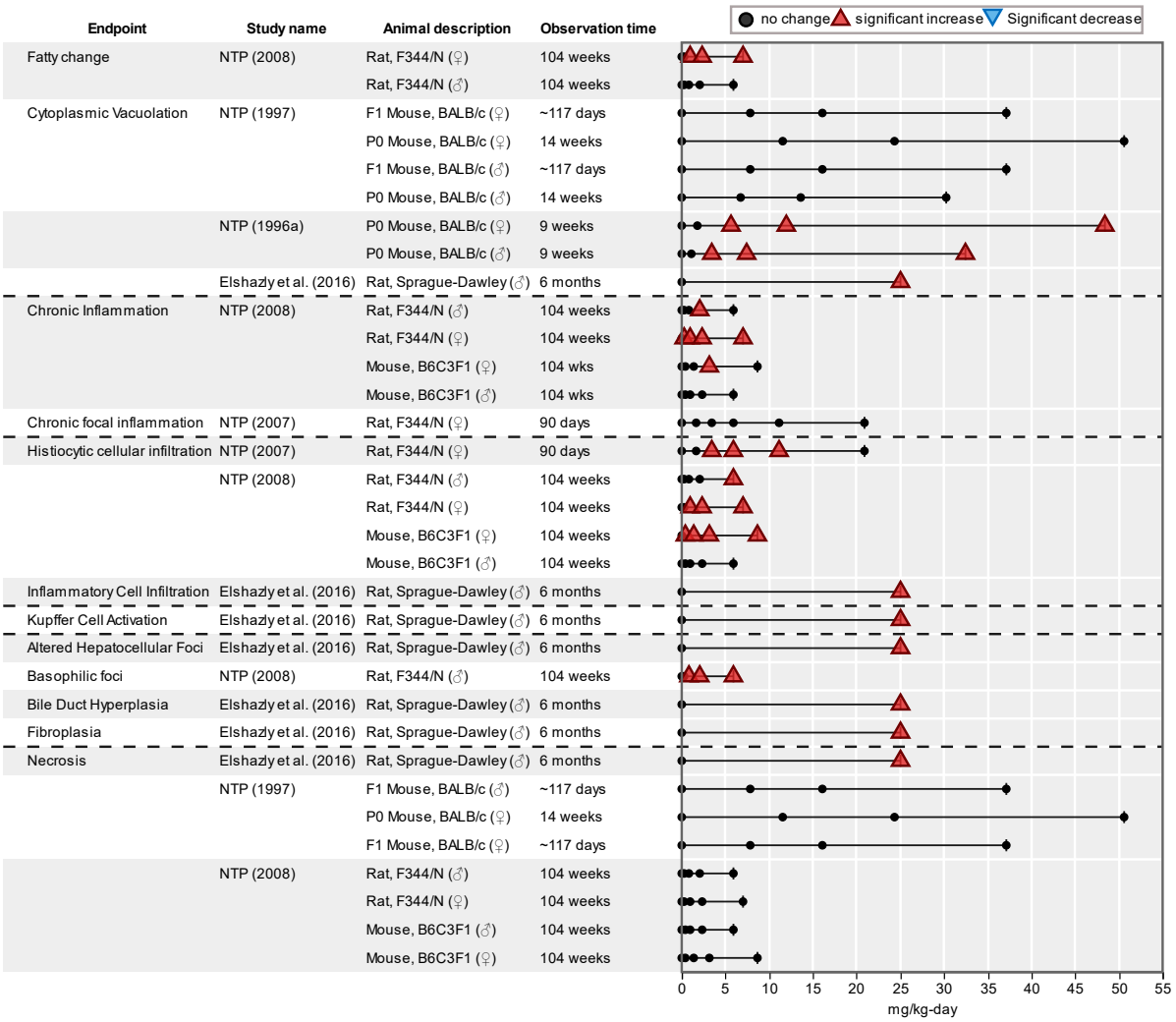

Figure 3-19. Hepatic effects of oral Cr(VI) exposure in animals (histopathology). Click to see an interactive graphic.

Clinical Chemistry

Many studies have examined serum indicators that are potentially informative for predicting hepatotoxicity following exposure to Cr(VI) (see Figure 3-20). The most commonly reported indicators included ALT, AST, ALP, and sorbitol dehydrogenase (SDH). ALT, in particular, is found abundantly in the cytosol of the hepatocyte, thus, in the case of hepatocellular injury, necrosis, or reparative activity, ALT is released into the bloodstream (Kim et al., 2008; Boone et al., 2005). Several studies by the oral route reported statistically significant increases in serum enzymes; however, no statistically or biologically significant increases in serum enzyme activities were observed in the available inhalation studies (Kim et al., 2004; Glaser et al., 1986; Glaser et al., 1985).

Statistically significant increases in ALT were reported in most of the studies in rats that measured this enzyme; increases ≥100% of the control mean were reported in approximately half

of these studies (Elshazly et al., 2016; NTP, 2008, 2007; Rafael et al., 2007; Acharya et al., 2001; Chopra et al., 1996). An increase in ALT of >100% (of the control mean) generally raises concern for hepatic injury (EMEA, 2008; Boone et al., 2005) and is considered biologically relevant. Biologically significant increases in ALT (>100%) were observed across studies in F344 and Wistar rats that were exposed to Cr(VI) for durations ranging from three months to two years at doses as low as 1–2 mg/kg-day (NTP, 2008, 2007; Acharya et al., 2001; Chopra et al., 1996). ALT was also statistically significantly elevated in some strains of mice following three months of exposure; however, these increases were smaller in magnitude (<100% of control) (NTP, 2007). Click here to see the magnitude of ALT changes in HAWC for NTP (2008, 2007).

Statistically significant increases in AST were also observed across rat studies (of various subchronic durations), with the magnitude of increase ranging from 60–113% above control mean (Navya et al., 2017a; Krim et al., 2013; Soudani et al., 2013; Acharya et al., 2001; Chopra et al., 1996; Meenakshi et al., 1989). However, many studies in the evidence base did not measure AST, including the *high* confidence NTP bioassays. AST is considered a less specific and sensitive indicator of hepatocellular injury than ALT (EMEA, 2008; Boone et al., 2005).

Increases in ALP, an indication of hepatobiliary damage (Boone et al., 2005), were less consistent across the evidence base, with some studies noting significant increases and other studies noting decreases in ALP. Several *high* confidence studies reported small (10–31%) but statistically significant decreases in ALP in F344 rats (NTP, 2008, 2007) and in one strain of male mice (NTP, 2007). However, decreases in ALP are not seen as a reflection of hepatobiliary toxicity, but are thought to be related to decreased food consumption (Travlos et al., 1996) or conditions including malnutrition, mineral deficiencies, and anemia (Lum, 1995), a finding noted in the NTP studies (2008, 2007). Four *medium* or *low* confidence studies in rats found statistically significant increases in ALP of 59–165% (Navya et al., 2017a; Elshazly et al., 2016; Krim et al., 2013; Chopra et al., 1996). An increase in ALP was noted in male Wistar rats exposed to 5.3–10.6 mg Cr(VI)/kg-day for 28–30 days (Navya et al., 2017a; Krim et al., 2013) and in female Wistar rats treated with 1.4 mg Cr(VI)/kg-day for 5.5 months (Chopra et al., 1996). No change relative to control was seen in male Wistar rats exposed to 1.4 mg Cr(VI)/kg-day for 5.5 months (Acharya et al., 2001).

Sorbitol dehydrogenase (SDH), considered to be a supplemental indicator of hepatotoxicity (Boone et al., 2005), was evaluated in two NTP studies (NTP, 2008, 2007). NTP reported statistically significant increases in SDH of 77–458% compared to controls in F344 male and female rats exposed to ≥1.7 mg Cr(VI)/kg-day for 3 months (NTP, 2007). Changes in SDH, in male rats only, were also observed in a 2-year NTP study conducted in the same rat strain that examined clinical chemistry endpoints at 3, 6, and 12 months (NTP, 2008). This study found more muted responses than the 3-month study (NTP, 2007), with statistically increased levels of SDH (24–69%) in the top two dose groups at the 6-month time point, but not at the 3- or 12-month time points (NTP, 2008). In mice, small but statistically significant decreases in SDH were observed in two strains of mice; however, decreases in SDH are not indicative of liver damage (NTP, 2007).

Hepatic glycogen levels may be affected by exposure to hepatotoxic chemicals. In animals exposed to Cr(VI), glycogen depletion was noted in two strains of male mice (NTP, 2007) and in two related studies in male and female Wistar rats (Acharya et al., 2001; Chopra et al., 1996). In NTP (2007), two strains of mice examined histologically showed glycogen depletion at doses ≥5.2 mg Cr(VI)/kg-day (B6C3F1) and ≥2.8 mg Cr(VI)/kg-day (*am3*-C57BL/6) but no glycogen depletion was found in exposed BALB/c mice (NTP, 2007). Acharya et al. (2001) and Chopra et al. (1996) also noted statistically significant decreased liver glycogen in rats exposed at 1.4 mg Cr(VI)/kg-day (the only dose tested) for 5.5 months. Hepatic glycogen levels are also dependent on caloric intake. NTP (2007) noted that the glycogen depletion was likely a result of depressed food consumption, often observed when water consumption is decreased, as it was at the high dose in this study; however, food consumption data was not reported.

Overall, significant increases in serum markers of liver damage were reported in several *high* and *medium* confidence oral exposure studies. Generally consistent elevations of ALT and AST were seen across multiple well-conducted studies in both rats and mice, with the magnitude of change in ALT considered to be biologically significant and a specific indication of liver damage. Changes to ALP and SDH were inconsistent across the evidence base and the biological significance of decreased glycogen observed in several studies is difficult to interpret. No effects on serum markers of liver damage were reported following inhalation exposures.

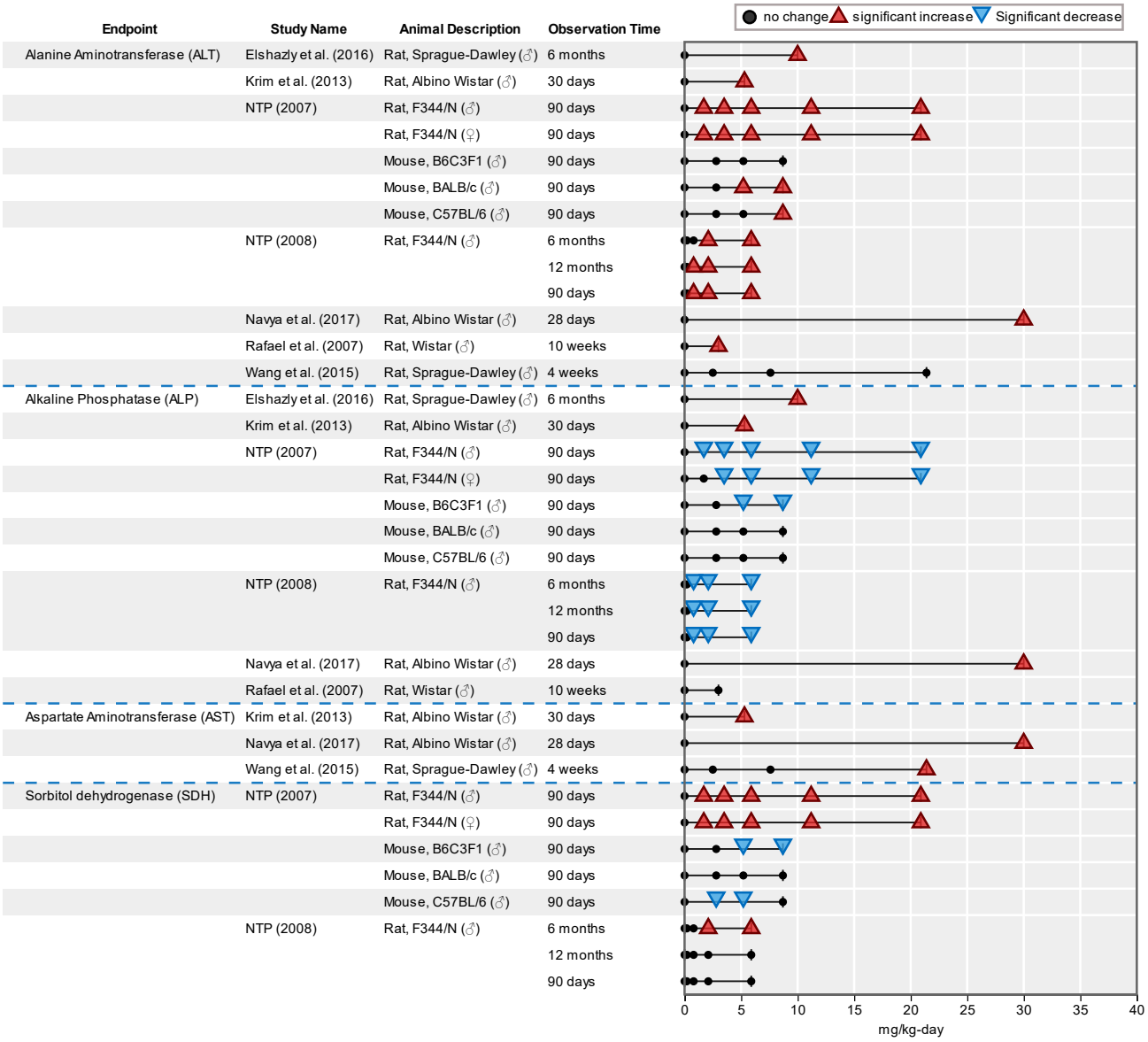

Figure 3-20. Hepatic effects of oral Cr(VI) exposure in animals (clinical chemistry). Click to see interactive graphic. To view the magnitude of changes in ALT from NTP (**2008**, **2007**) data, click here. To view data by **Elshazly et al. (2016)** (where dose could not be estimated), click here.

Liver weight

Several studies reported statistically significant changes (both increases and decreases) in absolute and relative liver weight (see Figure 3-21) following short-term or subchronic oral exposures; liver weight was not measured in the 2-year NTP (2008) bioassay. Liver weight relative to body weight has been shown to be more informative in the evaluation of liver toxicity, as compared to absolute liver weight, especially when changes in body weight are observed (Bailey et al., 2004). Therefore, this discussion focuses on changes in relative liver weight where available.

In the only high confidence study in rats, relative liver weights were decreased by about 10% in F344 males exposed to Cr(VI) in drinking water for three months in the two highest dose groups (11.2 and 20.9 mg Cr(VI)/kg-day) compared with control values; no significant liver weight changes were found in any female exposed group (NTP, 2007). Relative liver weight was substantially increased (>twofold) in female Wistar rats exposed to 1.4 mg Cr(VI)/kg-day in drinking water for 22 weeks in a low confidence study (Chopra et al., 1996). A shorter duration *medium* confidence study (4 weeks) in male Sprague-Dawley rats at doses up to 21 mg Cr(VI)/kg-day reported no change in liver weight (Wang et al., 2015).

In mice, several *high* confidence experiments conducted by NTP across three different strains observed a consistent pattern of absolute, but not relative, liver weight changes in high dose animals (9–30 mg Cr(VI)/kg-day) exposed to Cr(VI) through drinking water for about 3 months. Statistically significant decreases in absolute liver weights, but not relative liver weight, were observed in B6C3F1, BALB/c and *am3*-C57BL/6 mice (NTP, 2008, 2007). However, study authors reported that changes in absolute liver weight in these studies were correlated with decreased body weights seen at higher doses (NTP, 2008, 2007). Several older NTP studies in BALB/c mice did not measure liver weight (NTP, 1997, 1996a).

Regarding inhalation exposure, no changes in relative liver weight were observed in two 90-day rat studies at concentrations of 0.2 mg Cr(VI)/m^3 (Glaser et al., 1985) or 1.25 mg Cr(VI)/m^3 (Kim et al., 2004); however, an 18-month study at concentrations of up to 0.1 mg Cr(VI)/m^3 observed a statistically significant increase (13.5%) in relative liver weight (Glaser et al., 1986).

Overall, inconsistent findings were observed for relative liver weight changes in *high* and *medium* confidence oral exposure and *low* confidence inhalation studies, with decreases in relative liver weight observed in *high* confidence studies, and evidence for increased liver weight primarily limited to the *low* confidence studies.

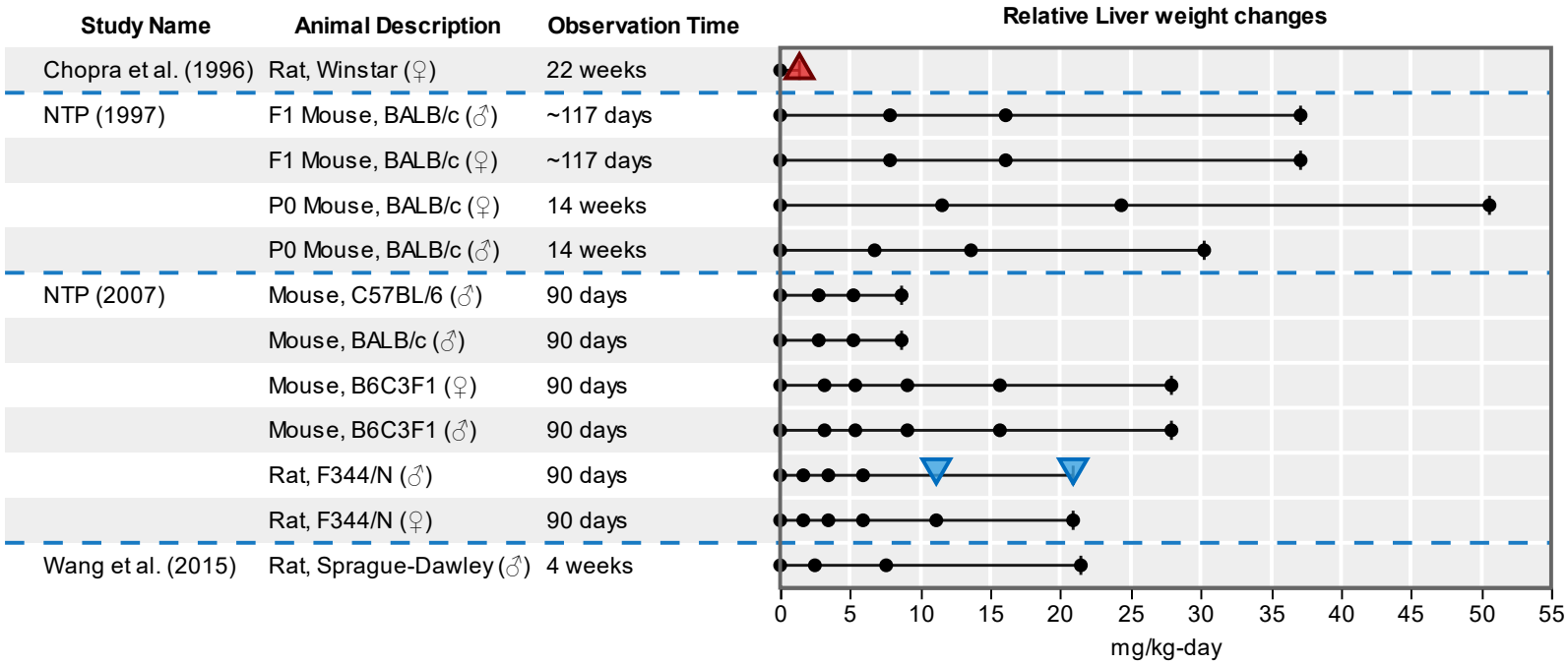

Figure 3-21. Hepatic effects of oral Cr(VI) exposure in animals (relative liver weight). Click to see an interactive graphic.

3.2.4.3. *Mechanistic Evidence*

The mechanistic data for liver toxicity indicates that several key events contribute to the hepatic effects observed in humans and animals. Exposure to Cr(VI) may cause oxidative and endoplasmic reticulum stress and mitochondrial dysfunction. These events can lead to inflammation and apoptosis, which can account for histopathological and serum indicators of liver injury seen in animals. In vivo experiments in rodents report that ingested and (to a lesser extent) inhaled Cr(VI) can accumulate in the liver (Jin et al., 2014; Cheng et al., 2000), demonstrating the metal can reach the target tissue and further supporting the biological plausibility for Cr(VI)-induced liver toxicity. A pharmacokinetic study by O'Flaherty and Radike (1991) demonstrated that following inhalation or oral exposure to nearly equivalent target absorbed doses of Cr(VI), oral exposure resulted in liver concentrations that were 1–2 orders of magnitude higher than those from inhalation exposure (See Appendix C.1.2). As a result, the extent of hepatotoxicity would be expected to differ by route of exposure.

A large body of mechanistic information (125 studies) exists to inform the potential hepatotoxicity of Cr(VI) (see Appendix C.2.3). Therefore, studies which are more informative for chronic human exposure were prioritized for further analysis and interpretation. These included mammalian studies that focused on exposure routes more relevant to humans (e.g., oral and inhalation studies), as well as repeat dose studies of longer durations (≥28 days). Shorter duration studies utilizing oral and inhalation routes of administration and in vitro studies in human cell lines also provided insight into biological plausibility and human relevance of the observed mechanisms.

Oral repeat dose studies provide support for oxidative stress, mitochondrial damage, inflammation, and apoptosis as mechanisms of Cr(VI)-induced liver effects. A 36-day dietary study in male mice receiving 1 and 4 mg/kg/$K_2Cr_2O_7$-day (0.35 and 1.41 mg/kg-d Cr[VI]) reported significant increases in hepatic lipid peroxidation and other markers of ROS-related stress (Jin et al., 2014), similar to a 10 week gavage study in rabbits receiving 5 mg/kg-day (El-Demerdash et al., 2006). Rafael et al. (2007) described immunohistochemical evidence for increased expression of Caspase-3, a marker for apoptosis in Wistar rats exposed to approximately 3 mg Cr(VI)/kg-day for 10 weeks. A 28-day study in male rats receiving 30 mg/kg/$K_2Cr_2O_7$-day (10.6 mg/kg-d Cr[VI]) by gavage (Navya et al., 2017a; Navya et al., 2017b) also reported increases in lipid peroxidation and decreased SOD, CAT, and GST activity, concurrent with increases in serum indicators of liver toxicity (ALT, AST, and ALP) and histological changes in the liver (described as feathery degeneration). These effects were concurrent with the upregulation of some genes involved in oxidative stress, inflammation, and apoptosis, such as TNF-α, MAPK, Atf-1, GADD-45, Bax, and Caspase-1, while anti-apoptotic genes, including Bcl-2 and OGG-1, were downregulated (Navya et al., 2017a; Navya et al., 2017b). Ninety- and 120-day studies in rats exposed to $Na_2Cr_2O_7$ (3.97 mg Cr(VI)/kg-day and 0.99 mg Cr(VI)/kg-day, respectively) reported lipid peroxidation in hepatic mitochondria and microsomes accompanied by increased urinary excretion of metabolites indicative of lipid peroxidation such as MDA (Bagchi et al., 1997; Bagchi et al., 1995a).

Oral studies in rats and mice of shorter, acute durations provide further support for a mode of action for Cr(VI)-induced liver effects involving oxidative stress and apoptosis. Similar to longer term repeat dose studies, shorter term and single-dose studies report increased chromium content in the liver, increased lipid peroxidation and ALT and AST, free radical production, indicators of inflammation, upregulation of pro-apoptotic genes and proteins, and down-regulation of anti-apoptotic genes and proteins in liver tissue (Zhong et al., 2017c; Wang et al., 2010c; Bagchi et al., 2002; Bagchi et al., 2001; Bagchi et al., 2000; Bagchi et al., 1995b; Kumar and Rana, 1982).

In vitro studies in human cell lines provide additional support for the biological plausibility of these liver toxicity mechanisms in humans. Human liver carcinoma cell lines show increases in ROS production and MDA at various concentrations as well as effects on antioxidant enzymes and mitochondrial function (Zhong et al., 2017a; Zeng et al., 2013; Patlolla et al., 2009). Similar results were observed in human fetal hepatocytes including increased mitochondrial stress, ER stress-related mechanisms, and the activation of apoptotic and senescence signaling cascades (Liang et al., 2019; Xiao et al., 2019; Zhang et al., 2019; Liang et al., 2018a; Liang et al., 2018b; Yi et al., 2017; Zhang et al., 2017; Zhong et al., 2017b; Zhong et al., 2017c; Zhang et al., 2016; Xiao et al., 2014; Xie et al., 2014; Xiao et al., 2012a; Xiao et al., 2012b; Yuan et al., 2012b; Yuan et al., 2012a). In vitro study results also support the upregulation of pro-inflammatory cytokines and signaling molecules such as NF-ƙB, TNF-α, LBT4, and IL1β (Zhong et al., 2017c; Yi et al., 2016).

Collectively, the data indicate oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis as possible interconnected mechanisms for liver toxicity. The toxicological evidence in animals taken together with mechanistic evidence, particularly data from oral, in vivo studies suggest a possible MOA of Cr(VI)-induced liver toxicity involving the production of free radicals and reactive intermediates through intracellular Cr(VI) reduction. In this possible MOA, the production of these reactive species alters antioxidant enzyme activity and stresses the endoplasmic reticulum and mitochondria, triggering an apoptotic signaling cascade. Oxidative stress may lead to liver inflammation and the upregulation of genes involved in an inflammatory response.

3.2.4.4. *Integration of Evidence*

Overall, the available **evidence indicates** that Cr(VI) likely causes hepatic effects in humans under relevant exposure circumstances. This conclusion is based on studies in animals that observed hepatic effects with drinking water exposure levels as low as 0.24–1.7 mg/kg-day Cr(VI). The human evidence for Cr(VI)-induced liver effects is limited in terms of number and confidence of studies. However, two of the available three studies (one occupational and one general population study) provide an indication of exposure-related alterations of liver clinical chemistry (Sazakli et al., 2014; Saraswathy and Usharani, 2007). Given the plausible support for these findings from in vitro studies of human hepatic cells, the human evidence is interpreted to provide *slight* evidence of hepatic toxicity associated with hexavalent chromium. Integrated evidence of the hepatic effects of Cr(VI) exposure from human, animal, and mechanistic studies is summarized in an evidence profile table, Table 3-26.

The available animal studies provide *moderate* evidence for liver effects in rats and mice orally exposed to Cr(VI) compounds, based primarily on elevated serum enzymes suggestive of liver toxicity, as well as histological evidence of inflammatory effects and fatty changes in the liver that are supported by a large and coherent database of in vivo mechanistic studies. This conclusion is specific to oral exposure to Cr(VI) as few, lower confidence inhalation studies evaluated liver toxicity and were generally null, possibly owing to the known differences in pharmacokinetics across routes.

Elevations of ALT and AST were seen across the oral evidence base, with biologically significant elevations in ALT (>100%) seen in multiple studies. ALT in particular is considered a sensitive and specific indicator of liver injury (Kim et al., 2008; Boone et al., 2005). Increased ALT is roughly correlated with the degree of hepatic inflammation, with patients with high ALT levels tending to have more severe inflammation in the liver than those with normal ALT values (Kim et al., 2008).

Chronic inflammation in the liver is a concern as it can lead to liver fibrosis (Koyama and Brenner, 2017). Dose-dependent increases in chronic inflammation were most evident in female F344 rats exposed for three months to two years (NTP, 2008). Lesser increases in chronic inflammation were also seen in male F344 rats and female (but not male) B6C3F1 mice exposed for two years, although background incidence of this lesion was high (NTP, 2008, 2007).

Fatty change (steatosis) is a common pathological change associated with liver disease, often leading to, or coinciding with, inflammation. If the insult responsible for steatosis persists, more severe pathologies can develop, including fibrosis and cirrhosis (Kaiser et al., 2012; Day and James, 1998). Histological findings of vacuolation and fatty changes were also observed in several studies (NTP, 2008; Acharya et al., 2001; Chopra et al., 1996; NTP, 1996a). Fatty changes are thought to be mediated by impaired mitochondrial function, which was observed in several studies of Cr(VI) exposure to human hepatic cells in vitro (Yi et al., 2017; Zhong et al., 2017c; Zhong et al., 2017a; Zhang et al., 2016; Xiao et al., 2014; Xie et al., 2014; Zeng et al., 2013; Xiao et al., 2012a; Yuan et al., 2012a; Patlolla et al., 2009).

Severe histological changes such as necrosis and fibrosis were not observed in the high-confidence NTP three-month or two-year studies in F344 rats and B6C3F1 mice (NTP, 2008, 2007). However, several lower confidence subchronic studies in rats noted increased evidence of apoptosis or necrosis (Elshazly et al., 2016; Rafael et al., 2007; Acharya et al., 2001; Chopra et al., 1996). These effects are supported by mechanistic evidence that suggests a possible MOA of Cr(VI)-induced liver toxicity involving the production of free radicals and reactive intermediates through intracellular Cr(VI) reduction resulting in oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis. Taken together, the serum enzyme and histopathology data from human, animal, and in vitro studies support biologically significant changes in the livers of rodents orally exposed to Cr(VI).

Table 3-26. Evidence profile table for hepatic effects

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
Evidence from studies of exposed humans					
CLINICAL CHEMISTRY **Low confidence:** Sazakli et al. (2014) Saraswathy and Usharani (2007) Lin et al. (1994)	Statistically significant changes in at least one marker of liver dysfunction (ALT, AST, ALP, bilirubin or total protein) were reported in 2 out of 3 *low* confidence studies, though the direction of the associations was not coherent for all endpoints across studies (i.e., increases in ALT, AST, ALP, and bilirubin would be expected to accompany decreases in total protein, but this was not consistently the case).	• Exposure-response gradient between exposure groups in one study for ALT, AST, ALP, and TP	• Lack of coherence of results across endpoints in some studies	⊕⊙⊙ *Slight* Although two studies reported changes in clinical chemistry markers of liver dysfunction, all studies were low confidence.	⊕⊕⊙ The **evidence indicates** that hexavalent chromium is likely to cause liver toxicity in humans. Effects on clinical chemistry were observed in both human and animal studies.
Evidence from animal studies					
HISTOPATHOLOGY (Oral) **High confidence:** NTP (1996a) NTP (1997) NTP (2007) NTP (2008)	Increased chronic inflammation and histiocyte infiltration with subchronic and chronic exposures in male and female rats and mice Increased fatty change and vacuolation	• Mostly *high* and *medium* confidence studies • Consistent findings regarding inflammatory changes and fatty changes/vacuolation across species and sexes		⊕⊕⊙ *Moderate* Findings of histopathological changes (particularly inflammation-related effects and fatty changes/vacuolation)	*Moderate* evidence in rats and mice shows consistent findings of elevated liver enzymes indicative of hepatocellular damage and changes in liver architecture following oral exposure. Mechanistic findings in animals provide evidence supportive of histopathological endpoints in the liver.

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
Medium confidence: Wang et al. (2015) Elshazly et al. (2016) **Low confidence:** Acharya et al. (2001) Chopra et al. (1996) Rafael et al. (2007)	No increase in necrosis in *high* confidence studies (apoptosis not evaluated); however, lower confidence studies and numerous mechanistic studies have indicated an increase in apoptosis and markers of apoptosis.	• Coherence with increases in ALT and AST • Mechanistic evidence provides biological plausibility		coupled with significant increases in ALT and AST are considered to be adverse and a specific indication of liver injury.	Oxidative stress was identified as a potential mechanism for liver effects in multiple animal species. This mechanism is presumed relevant to humans.
HISTOPATHOLOGY (Inhalation) **Medium confidence:** Kim et al. (2004) **Low confidence:** Glaser et al. (1985)	No histological changes in rats treated for 12 weeks or 18 months			Hepatic effects were generally not observed following inhalation exposures.	Hepatic effects were inconsistent following inhalation. Because of the first-pass effect, the liver may be affected more severely by Cr(VI) exposure via the oral route as compared to the inhalation route.
CLINICAL CHEMISTRY (Oral) **High confidence:** Krim et al. (2013) NTP (2007) NTP (2008)	Statistically significant elevations of ALT and AST seen across studies	• Consistent increases in ALT and AST in High and Medium confidence studies • Magnitude of effect- large effect size for ALT and AST	• Concerns for bias and sensitivity in all studies • Inconsistent findings		

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
Medium confidence: Navya et al. (2017a) Rafael et al. (2007) Wang et al. (2015) Elshazly et al. (2016) **Low confidence:** Acharya et al. (2001) Chopra et al. (1996) Meenakshi et al. (1989)	Biologically significant increases in ALT (>100%) were observed across studies and at doses as low as 1–2 mg/kg-day Changes to ALP were less consistent across the evidence base	• Dose-response gradient within studies • Coherence with histopathology (inflammation and fatty changes) • Mechanistic evidence of oxidative stress provides biological plausibility			
CLINICAL CHEMISTRY (Inhalation) **Medium confidence:** Kim et al. (2004) **Low confidence:** Glaser et al. (1985) Glaser et al. (1986)	No significant changes in enzymatic markers of liver damage (ALT, AST, ALP, SDH) following inhalation				

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
ORGAN WEIGHT (Oral) **High confidence:** **NTP (2007)** **NTP (1997)** **Medium confidence:** **Chopra et al. (1996)** **Wang et al. (2015)**	Inconsistent findings for relative liver weight changes in *high* and *medium* confidence oral studies, with no change or decreased relative liver weight observed in high and medium confidence studies and evidence for increased relative liver weight primarily limited to *low* confidence studies. Increases in absolute liver weight likely correlated with body weight decreases seen at high doses.		• Inconsistent findings in relative liver weight across studies of varying confidence levels.		
ORGAN WEIGHT (Inhalation) **Medium confidence:** **Kim et al. (2004)** **Low confidence:** **Glaser et al. (1985)** **Glaser et al. (1986)**	Changes in liver weight were inconsistent following inhalation exposures. One 18 month study observed a statistically and biologically significant (>10%) increase in relative liver weight (Glaser et al., 1986).				

Evidence summary and interpretation			
Mechanistic evidence			
Biological events or pathways	**Summary of key findings and interpretations**	**Judgments and rationale**	**Inferences and summary judgment**
Oxidative and endoplasmic reticulum stress	*Interpretation:* Consistent in vivo and in vitro evidence of Cr(VI)-induced oxidative and ER stress evidenced by increased lipid peroxidation, ROS, and decreased antioxidant enzyme activity concurrent with biomarkers of liver injury. *Key findings:* • Consistent evidence of significant increases in lipid peroxidation in liver tissue in chronic, subchronic and acute dose animal studies (Navya et al., 2017a; Zhong et al., 2017c; Jin et al., 2014; Wang et al., 2010c; Bagchi et al., 2002; Bagchi et al., 2001; Bagchi et al., 2000; Bagchi et al., 1997; Bagchi et al., 1995b; Bagchi et al., 1995a; Kumar and Rana, 1982) • Increased oxidative stress (decreased antioxidant enzyme activity) concurrent with serum biomarkers of liver injury (increased ALT, AST, and ALP) in a 28-day study in rats (Navya et al., 2017a) • Increased oxidative stress (lipid peroxidation, free radical production) concurrent with serum biomarkers of liver injury (increased ALT and AST) in liver tissue in short-term and acute oral exposure studies in rats and mice (Zhong et al., 2017c; Wang et al., 2010c; Bagchi et al., 2002; Bagchi et al., 2001; Bagchi et al., 2000; Bagchi et al., 1995b; Kumar and Rana, 1982) • In vitro evidence of increased ROS production and MDA and effects on antioxidant enzymes in human liver carcinoma cell lines (Zhong et al., 2017a; Zeng et al., 2013; Patlolla et al., 2009) • In vitro evidence of ER stress-related mechanisms in human cells (Zhang et al., 2017)	Biologically plausible, consistent, coherent observations of oxidative stress and endoplasmic reticulum stress, mitochondrial dysfunction, inflammation, and apoptosis concurrent with apical observations of liver toxicity following (oral) exposures to Cr(VI) in animals, supported by in vitro evidence in human cells.	

Evidence summary and interpretation			
Mechanistic evidence			
Biological events or pathways	**Summary of key findings and interpretations**	**Judgments and rationale**	**Inferences and summary judgment**
Mitochondrial dysfunction	*Interpretation:* In vitro evidence in human liver cell lines of Cr(VI)-induced mitochondrial dysfunction. *Key findings:* • In vitro evidence of effects on mitochondrial function in human liver carcinoma cell lines (Zhong et al., 2017a; Zeng et al., 2013; Patlolla et al., 2009) • In vitro evidence of increased mitochondrial stress in human fetal hepatocytes (Yi et al., 2017; Zhong et al., 2017c; Zhang et al., 2016; Xiao et al., 2014; Xie et al., 2014; Xiao et al., 2012a; Yuan et al., 2012a)		
Inflammation	*Interpretation:* Consistent in vivo and in vitro evidence of Cr(VI)-induced liver inflammation. *Key findings:* • Increased indicators of inflammation concurrent with serum biomarkers of liver injury (increased ALT and AST) in liver tissue in short-term and acute oral exposure studies in rats and mice (Zhong et al., 2017c; Wang et al., 2010c; Bagchi et al., 2002; Bagchi et al., 2001; Bagchi et al., 2000; Bagchi et al., 1995b; Kumar and Rana, 1982) • In vitro evidence of the upregulation of pro-inflammatory cytokines and signaling molecules such as NF-ĸB, TNF-α, LBT4, and IL1β in human cells (Zhong et al., 2017c; Yi et al., 2016)		

Evidence summary and interpretation			
Mechanistic evidence			
Biological events or pathways	**Summary of key findings and interpretations**	**Judgments and rationale**	**Inferences and summary judgment**
Apoptosis	*Interpretation:* Cr(VI) alters protein and gene expression of biomarkers associated with apoptosis in vivo concurrent with liver injury. *Key findings:* • Increased expression of caspase-3 and histological changes indicative of apoptosis in a 10-week rat study (Rafael et al., 2007) • Upregulated transcription of pro-apoptotic genes and downregulated transcription of anti-apoptotic genes concurrent with serum biomarkers of liver injury (increased ALT, AST, and ALP) in a 28-d rat study (Navya et al., 2017a) • Upregulation of pro-apoptotic genes and proteins and downregulation of anti-apoptotic genes and proteins concurrent with serum biomarkers of liver injury (increased ALT and AST) in liver tissue in short-term and acute oral exposure studies in rats and mice (Zhong et al., 2017c; Wang et al., 2010c; Bagchi et al., 2002; Bagchi et al., 2001; Bagchi et al., 2000; Bagchi et al., 1995b; Kumar and Rana, 1982) • In vitro evidence of the activation of apoptotic signaling cascades in human fetal hepatocytes (Yi et al., 2017; Zhong et al., 2017c; Zhang et al., 2016; Xiao et al., 2014; Xie et al., 2014; Xiao et al., 2012a; Yuan et al., 2012a)		

3.2.5. Hematologic effects

Hematology is a subgroup of clinical pathology concerned with morphology, physiology, and pathology of blood and blood-forming tissues. Hematology parameters routinely measured using blood tests such as complete blood count (CBC) and a clinical chemistry panel are described in Table 3-27. A first tier diagnostic test typically measures three primary blood types: red blood cells (RBCs), white blood cells, and platelets. These measures and other RBC indices are useful indicators of blood pathology, including anemia and leukemia. RBCs carry oxygen throughout the body, while white blood cells are involved in immune function (discussed in Section 3.2.6) and platelets are involved in blood clotting. RBCs also carry most of the body's iron, which can be indirectly measured in blood by measuring transferrin, a membrane-bound transporter of ferric (Fe^{+3}) iron, and total iron binding in blood. Hematology along with clinical chemistry measures (e.g., blood proteins, enzymes, chemicals and waste products) and other general health status indicators are useful for assessing overall health status, monitoring disease, and determining if follow-up testing is needed. In humans, hematology and clinical chemistry test results are interpreted according to reference range criteria to identify values outside of normal background ranges (summarized in Table 3-27).

RBCs act as a sink for chromium in the blood. Cr(VI) is rapidly taken up by RBCs, where it is reduced to Cr(III) and remains trapped for the lifetime of the cell (see Section 3.1 and Appendix C for more details). After RBCs are broken down, the Cr(III) is released to systemic circulation and may be absorbed by other tissues or excreted in urine. Because Cr(III) cannot readily cross cell membranes, the RBC chromium level is commonly used as a biomarker for Cr(VI) exposure in industrial settings (Miksche and Lewalter, 1997). The focus of this section is primarily on RBCs and related components. Cr(VI) effects on white blood cell parameters are discussed in the context of the immune system in Section 3.2.6.

Table 3-27. Hematologic endpoints commonly evaluated in routine blood testing

Endpoint	Description	Human Reference Range[a]	Animal Reference Range
Hemoglobin (Hgb, g/dL)	Iron-containing oxygen-transport metalloprotein in RBCs	Male: 13.5 to 17.5 Female: 12.0 to 15.5	Male: 14.4 ± 0.91[b] Female: 13.8 ± 1.09[b] Male: 15.9 ± 1.0[c] Female: 15.5 ± 1.0[c]
Hematocrit (Hct)	Percentage (by volume) of the blood that consists of RBCs Hematocrit (%) = MCV × RBC / 10	Male: 38.3 to 48.6% Female: 35.5 to 44.9%	Male: 39.1 ± 2.7[b] Female: 36.9 ± 2.96[b] Male: 47.0 ± 3.1[c] Female: 45.1 ± 2.8[c]

Endpoint	Description	Human Reference Range[a]	Animal Reference Range
Red blood cell (RBC; erythrocyte) count	The most common blood cell responsible for systemic oxygen delivery. Expressed as number of RBCs per µL of blood	Male: 4.35 to 5.65 million Female: 3.92 to 5.13 million	Male: 8.01 ± 0.59[b] Female: 7.03 ± 0.66[b] Male: 8.91 ± 0.75.3[c] Female: 8.17 SD 0.63[c]
Reticulocytes	Immature non-nucleated RBCs containing residual RNA; indicates rate of new RBC production. The normal range depends on your level of hemoglobin. Hemoglobin is a protein in red blood cells that carries oxygen. The range is higher if hemoglobin is low, from bleeding or if red cells are destroyed.	0.5 to 2.5	Male: 2.0 ± 1.23[b] Female: 1.6 ± 0.87[b] Male rat 1.9 ± 0.9[c] Female rat 1.8 ± 0.8[c]
Mean corpuscular (cell) volume (MCV)	Average volume of the RBC MCV = hematocrit × 10 / RBC Low MCV: microcytic (smaller RBCs, possibly caused by iron deficiency and anemia); high MCV: macrocytic (larger RBCs, possibly caused by excess iron).	80–100 fl	Male: 51.3 ± 3.0[b] Female: 52.6 ± 2.7[b]
Mean cell hemoglobin (MCH)	Average weight of hemoglobin (Hb) in the RBC MCH = Hb × 10 / RBC, (g/dL) Hemoglobin concentration normalized as amount of hemoglobin per cell. High MCH: may indicate macrocytic anemia (large red blood cell volume leading to low Hb concentration), while low MCH may indicate other types of anemia (e.g., from iron deficiency).	Male: 13.5 to 17.5 Female: 12.0 to 15.5	Male: 19.0 ± 1.9[b] Female: 19.8 ± 0.94[b]
Mean cell hemoglobin concentration (MCHC)	Average concentration of Hb in the RBC volume MCHC = Hb × 100 / hematocrit (g/dL) Hemoglobin concentration normalized to red blood cell volume. Low MCHC: hypochromic (RBCs paler than normal); high MCHC: hyperchromic (RBCs more pigmented than normal)	33.4–35.5	

[a]Human reference range values from Mayo Clinic
[b]Reference ranges from Crl:COBSCD Sprague-Dawley rats aged <6 months, n = 324 (Wolford et al., 1986)
[c]Reference ranges from Sprague-Dawley rats aged 10–20 weeks old, n = 2235–2816 (Matsuzawa et al., 1993)

3.2.5.1. *Human Evidence*

Study evaluation summary

There are five studies that reported on the association between Cr(VI) exposure and hematologic parameters pertaining to the erythron, specifically complete blood counts (CBC), including RBC, hemoglobin, hematocrit, and platelets. Four studies were classified as *low* confidence (Table 3-28). Sazakli et al. (2014) was limited due to exposure measurement; exposure was estimated using water intake and historic water concentration records as well as hair and blood concentrations. Correlations between these measures were low. It is likely that any exposure

misclassification would be nondifferential and therefore lower the precision of the effect estimates but is unlikely to bias the results away from the null. Sharma et al. (2012) was limited in most domains, and exposure was based on residence in a geographic area with contaminated groundwater, which does not distinguish the heterogeneity of exposure across exposed participants. Lacerda et al. (2019) was limited due to potential for selection bias and confounding and Song et al. (2012) was limited due to potential for confounding. The remaining study (Khan et al., 2013) was classified as *uninformative* because exposure was based on tannery work, and there was insufficient information provided on the specific tanning processes used at the facility[36].

Table 3-28. Summary of human studies for Cr(VI) hematologic effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.
Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Exposure Measurement	Study Design	Clinical Chemistry
Lacerda et al. (2019)	Chrome-plating workers	Brazil	Exposure group validated by urine, blood sampling	Cross-sectional	L
Sazakli et al. (2014)	General population	Greece	Urine, Hair, Modeled lifetime chromium exposure dose	Cross-sectional	L
Sharma et al. (2012)	General population	India	Residence in geographic area with contaminated groundwater vs. control	Cross-sectional	L
Song et al. (2012)	Chromate production workers	China	Work category validated by air, blood sampling	Cross-sectional	L
Khan et al. (2013)	Tannery	Pakistan	Blood, Urine, Work category	Cross-sectional	U

Synthesis of evidence in humans

One of the included *low* confidence studies (Sazakli et al., 2014) reported statistically significant decreases in hemoglobin and hematocrit (Table 3-29), inconsistent with another *low* confidence study that reported statistically significant increases in the same endpoints (Lacerda et al., 2019). Song et al. (2012) reported no association with hemoglobin but did not report on hematocrit. Another *low* confidence study reported higher red blood cell counts and lower mean corpuscular volume (MCV) in exposed participants, stratified by sex (all statistically significant except MCV in women) (Sharma et al., 2012). None of the other studies reported an association between Cr(VI) exposure and RBC count, and none examined associations with diagnosed anemia or dichotomized hematologic parameters as the outcome. Platelet findings were also inconsistent.

[36]Leather tanning processes that can potentially lead to Cr(VI) exposure include: 1) use of a two-bath process, 2) on-site production of tanning liquors, and 3) leather finishing steps that involve Cr(VI) (e.g., use of Cr(VI)-containing pigments) (Shaw Environmental, 2006). If these processes are not specified by the study, it cannot be determined whether exposure was to Cr(VI) or Cr(III).

Sharma et al. (2012) reported lower platelets in exposed participants, while Sazakli et al. (2014) reported higher platelets with higher exposure, both statistically significant.

Table 3-29. Associations between Cr(VI) and hematologic parameters in epidemiology studies

Reference, confidence	Population	Exposure comparison and effect estimate	RBC (10^{12}/L)	Hemoglobin (g/dL)	Hematocrit (%)
Lacerda et al. (2019), low	Cross-sectional in Brazil, chrome-plating workers (n = 50) and controls (n = 50)	Means ± SD for chromium unexposed/ exposed	Unexposed: 5.34 ± 0.79 Exposed: 5.95 ± 0.90	Unexposed: 14.16 ± 0.40 Exposed: 15.70 ± 0.14*	Unexposed: 39.18 ± 0.49 Exposed: 43.30 ± 0.36*
Sazakli et al. (2014), low	Cross-sectional in Greece, general population; Two exposure groups (n = 237) and controls (n = 67)	Regression coefficients for calculated lifetime exposure dose and Cr in hair	Lifetime: 0.007 Hair: −0.09	Lifetime: −0.09* Hair: −0.06	Lifetime: −0.09* Hair: −0.1*
Sharma et al. (2012), low	Cross-sectional in India, general population with residence in contaminated area (n = 186) or not (n = 230)	Means ± SD for chromium unexposed/ exposed	Males Unexposed: 4.28 ± 0.69 Exposed: 5.55 ± 1.39* Females Unexposed: 3.89 ± 0.71 Exposed: 5.67 ± 1.26*	NR	NR
Song et al. (2012), low	Cross-sectional in China, chromate production workers (n = 100) and controls (n = 80)	Means ± SD for chromium unexposed/ exposed	Unexposed: 4.7 ± 0.4 Exposed: 4.8 ± 0.8	Unexposed: 144.8 ± 12.6 Exposed: 148.8 ± 27.2	NR

*$p < 0.05$. Shading indicates results supportive of an association between Cr(VI) and hematologic parameters in the direction of anemia (i.e., decrease in red blood cells, hemoglobin, and hematocrit).
NR: not reported

Due to inconsistent results across *low* confidence studies, there is no clear evidence of an association between Cr(VI) exposure and hematologic effects in humans. There is not a clear explanation for the conflicting results in Lacerda et al. (2019) and Sazakli et al. (2014), even when considering differences in route of exposure. The null findings in Song et al. (2012) may be due to reduced sensitivity, but this is a very limited evidence base in terms of number and confidence of studies so further exploration of patterns by exposure levels/analysis are not possible.

3.2.5.2. *Animal Evidence*

Study evaluation summary

Table 3-30 provides a summary of the animal toxicology studies considered in the evaluation of the hematologic effects of Cr(VI). The available evidence included 14 studies conducted in rats (three strains) and mice (three strains). Exposure durations and routes included one chronic oral study (NTP, 2008), one subchronic oral study (NTP, 2007), seven oral 3–9 week studies (Wang et al., 2015; Krim et al., 2013; NTP, 2006a, b, 2005, 1996a, b), one study conducted using NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (NTP, 1997), and four inhalation studies ranging from short-term to chronic exposure durations (Kim et al., 2004; Glaser et al., 1990; Glaser et al., 1986; Glaser et al., 1985).

Of the 15 included studies, 10 were considered *medium* or *high* confidence studies, and included eight National Toxicology Program (NTP) studies with exposure durations ranging from 4 days to 12 months (Table 3-30). Three of the four inhalation studies and one of the 11 oral studies that examined hematologic endpoints were considered *low* confidence mostly because of limited reporting of study methods and/or results. Five additional studies with hematologic data were judged *uninformative* based on critical deficiencies identified when the studies were evaluated (i.e., Anwar et al. (1961) mixed animals of different breeds; Kumar and Barthwal (1991) did not use concurrent controls; Shrivastava et al. (2005a) lacked information on sex, number of mice, and control group; and Zabulyte et al. (2009) and Zabulyte et al. (2006) had multiple deficiencies including randomization procedures, lack of vehicle control, and others). Full study evaluation details are available in HAWC.

Table 3-30. Summary of included studies for Cr(VI) hematologic effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive data graphic for rating rationales.

Author (year)	Species (strain)	Exposure design	Exposure route	Hematologic outcomes[b]
NTP (1996a)	Mouse (BALB/c)	3, 6, and 9 wk	Oral (Diet)	H
NTP (1996b)	Rat (Sprague-Dawley)	3, 6, and 9 wk	Oral (Diet)	H
NTP (1997)	Mouse (BALB/c)	Continuous breeding design	Oral (Diet)	H
NTP (2005)	Mouse (B6C3F1), female	28 d	Oral (Drinking water)	H

Author (year)	Species (strain)	Exposure design	Exposure route	Hematologic outcomes[b]
NTP (2007)	Rat (F344/N) Mouse (B6C3F1) Mouse (B6C3F1, BALB/c, *am3*-C57BL/6), male—comparative toxicity study	90 d	Oral (Drinking water)	H
NTP (2008)	Rat (F344/N), male Mouse (B6C3F1), female	2 yr	Oral (Drinking water)	H
NTP (2006b)	Rat (Sprague-Dawley), female	28 d	Oral (Drinking water)	M
NTP (2006a)	Rat (F344), female	28 d	Oral (Drinking water)	M
Krim et al. (2013)	Rat (Wistar), male	30 d	Oral (Gavage)	M
Wang et al. (2015)	Rat (Sprague-Dawley), male	28 d	Oral (Drinking water)	M
Kim et al. (2004)	Rat (Sprague-Dawley), male	90 d	Inhalation	M
Glaser et al. (1985)	Rat (Wistar), male	28 and 90 d	Inhalation	L
Glaser et al. (1986)	Rat (Wistar), male	18 months	Inhalation	L
Glaser et al. (1990)	Rat (Wistar), male	30 and 90 d	Inhalation	L
Samuel et al. (2012a)	Rat (Wistar), female	Pregnant dams, GD 9–21	Oral (Drinking water)	L

[a]Studies in this table were ordered first by route of exposure, and then by confidence rating. Within a confidence rating, studies were ordered chronologically.

[b]Within each study, multiple hematologic outcomes (such as those in Table 3-27) were typically measured using analytical methods for complete blood counts. For this reason, multiple outcome sensitivity ratings are not presented.

Synthesis of evidence in animals

Evidence informing Cr(VI) effects on hematologic endpoints was available from several (mostly short-term) *medium* and *high* confidence oral exposure studies (Table 3-30). There were two *high* confidence studies, one subchronic (NTP, 2007) and one chronic (NTP, 2008) bioassay, reporting hematologic outcomes in F344 rats and B6C3F1 mice that were useful for evaluating the potential subchronic and lifetime hematologic effects of Cr(VI) exposure in humans. Both studies are discussed below in detail and are summarized in HAWC in an interactive visualization that is available by clicking the following link: Cr(VI) Hematology (NTP) and in Figure 3-22 below (note

that only observation times at 90 days and greater are presented).[37] Methodological considerations for evaluating hematology findings in general included alterations in water intake, fasted/fed status, lifestage, and sex. The findings were also considered along with available reference ranges to assess the biological significance of the observed changes.

[37]Older data from other *medium* and *high* confidence studies performed by the National Toxicology Program (NTP, 2006a, b, 2005, 1997, 1996a, b) are consistent with results by NTP (2008, 2007). Only the most recent NTP results are synthesized, because they provide data at a wide dose range for multiple species and strains, and also provide data from multiple timepoints within its 2-year study.

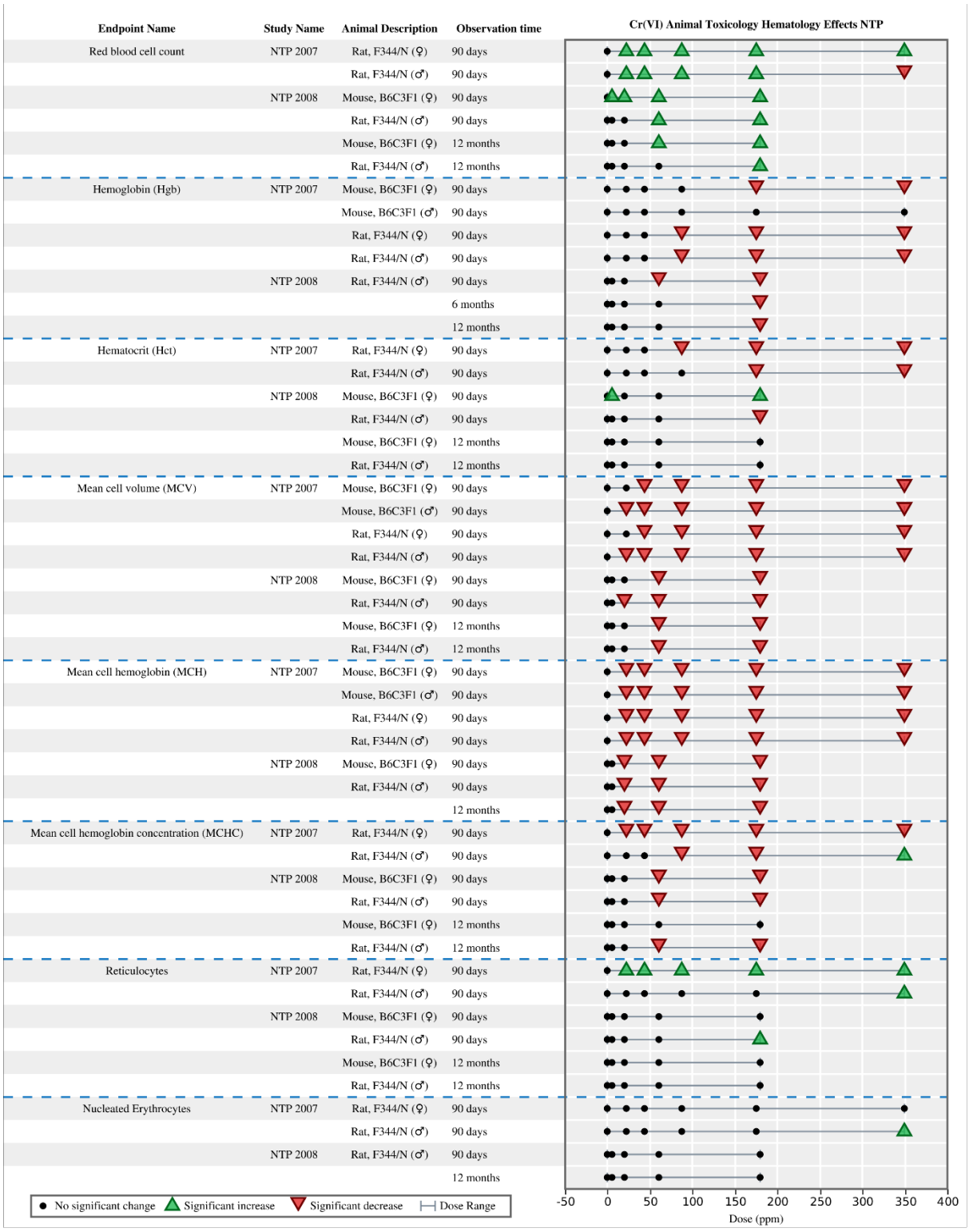

Figure 3-22. Hematology findings from NTP (2007) and NTP (2008) in rats and mice exposed by gavage to Cr(VI) for 90 days or 12 months (full details available in the HAWC link).

Direct measures of hematopoietic health include RBCs, hemoglobin (Hgb), and hematocrit (Hct) levels (see Table 3-27). RBCs were increased across study designs, sexes, and species in both a *high* confidence subchronic study (NTP, 2007) and a *high* confidence chronic bioassay (NTP, 2008) (Figure 3-22). Statistically significant treatment effects corresponded with an approximately 2–4% change in the 20 mg/L dose group, 4–8% change at 60 mg/L, and 5–18% change from controls in the 180 mg/L dose group (NTP, 2008) (click to view RBC findings)[38]. Note that RBC counts were greater at 90 days than 12 months within each dose group and sex. Hgb was decreased in both male and female rats and female mice at 9 and 12 month observation times at doses ≥174.5 mg/L. The magnitude of change was <5% from control mean for all findings except in the ≥174.5 mg/L dose groups. Hct increased in female mice at 90 days and decreased in male and female rats at doses ≥174.5 mg/L. No changes in Hct were observed in either species at 12 months Cr(VI) exposure.

In humans, it is important clinically to interpret RBCs, Hgb, and Hct findings in the context of reference ranges (i.e., historical controls) to determine if follow-up is needed. In animals, rat reference ranges were available from two studies of Sprague-Dawley rats (Matsuzawa et al., 1993; Wolford et al., 1986). Both studies reported one standard deviation from the mean that was equivalent to a ~6–8% change from the mean for RBC, Hgb, and Hct in 10–20 week old rats; however, there was significant strain variation in the population means, indicating there may be uncertainty in applying Sprague-Dawley rat references ranges to the F344 rats used in the NTP studies. Reference ranges for F344 rats were available from ToxRefDB (Watford et al., 2019), a repository for in vivo toxicity data, indicating an average variation of <4% for Hct and RBCs and <2% for Hgb. Interpretation of the NTP findings in the context of the Sprague-Dawley (Matsuzawa et al., 1993; Wolford et al., 1986) reference ranges suggests significant biological variation at 9 months that returns to normal by 12 months. However, interpretation of the NTP findings using the F344 (ToxRefDB) rat reference ranges suggests the observed collection of hematologic changes in rats at both 90 days and 12 month Cr(VI) exposures have potential biological significance, although the magnitude of the effect is low.

While the RBC, Hgb, and Hct findings were considered to be potentially adverse at 90 days based on *high* confidence studies, the magnitude of change, the increasing responses with dose, and consistency across species and sexes supported by coherent changes in other RBC indicators (MCV, MCH, and MCHC), the adversity of effects at 12 months were less certain and potentially adaptive. Decreased mean cell volume (MCV) values (i.e., smaller RBCs) were consistently observed across

[38]Exposures for NTP (2008) and NTP (2007) are expressed in the text as concentration in drinking water (mg Cr(VI)/L) rather than daily dose (in mg Cr(VI)/kg-day). Differences in rodent drinking water consumption rates relative to body weight during the growth period lead to different mg/kg-d doses at the different collection times within the same exposure group of the 2-year study. Discussion in units of drinking water concentrations simplifies the group-level comparisons. Estimates of time weighted average daily doses at different observation time are available here (for NTP (2008)) and here (for NTP (2007)). At 20 mg/L Cr(VI) in rats for the 2-year study, the time weighted average dose was 2 mg/kg-d at 22 days, 1.5 mg/kg-d at 90 days, and 0.88 mg/kg-d at 1 year.

study designs, sexes, and species (although male rats were the most sensitive) in both *high* confidence NTP bioassays (NTP, 2008, 2007), but while MCV decreases were dose-responsive across rat 90 day observation times, with a maximal response of a ~30% change from control in male rats receiving 349 mg/L for 90 days, when comparing the MCV response to Cr(VI) exposure from 90 days to 12 months, the 12 month response was less robust (23% decrease compared with 7% at 12 months). Cr(VI) effects on MCH were consistent and coherent with MCV; decreases were dose-responsive across 90 day and 12 month observation times, with a maximal response of ~30% at 349 mg/L (90 days), but similar to MCV, the response was less intense at 12 months (~8% decrease from control) compared with same dose at 90 day observation time (~27% change) in rats. The MCHC response to Cr(VI) exposure in rats and mice was muted compared with MCV and MCH, with a maximum response of 5–10% change from control in male and female rats exposed for 90 days to ≥174.5 mg/L. The dose-response was less clear at 12 months exposure. The pattern of response, however, was similar to MCH and MCV when comparing the MCHC response between exposure durations and species, with a greater response at 90 days compared to 12 months, and in rats compared with mice.

Reticulocytes (RET) and nucleated reticulocytes are immature RBCs and their levels may indicate alterations in RBC production (Whalan, 2015, 2000). Reticulocytes and nucleated erythrocytes were increased, but the finding was inconsistent across species and sexes with increases observed in males only at the maximum dose (NTP, 2008, 2007) and in all female dose groups at 90 days (NTP, 2007). Notably, the observed changes from control for reticulocyte measures (NTP, 2008, 2007) were within normal reference ranges reported by Matsuzawa et al. (1993) and Wolford et al. (1986). Microscopic evaluation of blood smears at exposure durations up to 90 days identified erythrocyte fragments and keratocytes (evidence of stress or damage to the bone marrow and evidence of increased RBC injury or turnover) (NTP, 2008, 2007). Similar microscopic findings from blood smears were not observed after 12 months Cr(VI) exposure.

Although the focus of the assessment is on the development of chronic reference values, hematologic effects were observed in studies with exposure durations <90 days. In general, the direction of change was similar to the later time points, but the magnitude of response was greater at observation times <90 days, peaking at 22 days (NTP, 2008). Other *medium* and *high* confidence studies were also available at exposure durations ≤9 weeks. In general, these studies reported limited or no statistically significant changes in hematologic parameters at the same dose levels where effects were observed in the subchronic and chronic studies. Decreased MCV and MCH levels (≤6%) were observed in Sprague-Dawley rats exposed to ≥10 mg Cr(VI)/kg-day (via diet) for up to 9 weeks (NTP, 1996b). In two other 28-day studies by NTP, hematologic effects at doses ≥9 mg Cr(VI)/kg-day exposure (via drinking water) were not observed for RBCs, hemoglobin, hematocrit, and MCHC in female Sprague-Dawley and F344 rats (NTP, 2006a, b). MCV and MCH findings were not dose responsive nor considered biologically meaningful.

The hematologic effects of inhalation exposure were reported in one *medium* confidence

study (Kim et al., 2004) where findings included increased RBC count (8%), decreased hematocrit (≤11%), and decreased hemoglobin (≤8%) in Sprague-Dawley rats exposed for 90 days to Cr(VI) concentrations ranging from 0.2–1.25 mg/m^3. No effects on MCV or MCHC were observed. No effects on RBCs were reported in male Wistar rats in three *low* confidence studies with exposure durations that ranged from 28 days to 18 months (Glaser et al., 1990; Glaser et al., 1986; Glaser et al., 1985), whereas the 30- and 90-day experiments did not specify which hematologic parameters were examined. The highest concentrations tested ranged from 0.1–0.4 mg/m^3; the highest concentration tested in the 18-month study by Glaser et al. (1986) (0.1 mg/m^3) was lower than the lowest concentration tested by Kim et al. (2004) (0.2 mg/m^3).

3.2.5.3. *Mechanistic Evidence*

The subchronic and chronic studies provide evidence for microcytic hypochromic anemia (characterized by low Hgb concentrations in abnormally small RBCs) after 90 days. After 12 months exposure, most findings returned to near normal levels (Hgb, Hct, MCHC) or were potentially in the low-normal range (RBC, MCV, MCH) when comparing to rat reference ranges. The clinical pathology and microscopic evaluation indicated small RBCs (microcytic) that were hypochromic (less color consistent with decreased Hgb). The mechanistic studies provide evidence for connecting these findings to upstream events, including altered iron metabolism leading to iron deficiency, and oxidative stress potentially leading to RBC damage, smaller size, and increased turnover.

Effects on iron homeostasis

Iron is a critical requirement for metabolic processes including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour et al., 2014). Iron imbalance, deficiency, and overload have known health effects in humans including iron-deficient anemia and iron toxicity. Iron is abosorbed from the diet by villous enterocytes in the small intestine. Cellular iron import involves both receptor-mediated endocytosis (by transferrin) of ferric iron (Fe^{+3}) as well as uptake of reduced iron ferrous iron (Fe^{2}) by membrane-bound transporters. A majority of the iron is contained by RBCs where iron is stored in complexes with ferritin (in the ferric state), complexed by heme in the ferrous state (Fe^{2}+), or to a smaller extent labile in the cytosolic pool in the ferrous state (Fe^{2}+). Several studies provided evidence that Cr(VI) intereferes with iron homeostasis, thereby decreasing iron bioavailability. Although blood iron measures were not available from the NTP studies, a subchronic study by Suh et al. (2014) reported a dose-responsive reduction in iron levels in serum, duodenum, liver, and bone marrow in F344 rats and B6C3F1 mice administered Cr(VI) (as sodium dichromate dihydrate) in drinking water for 90 days (0.1–180 mg Cr(VI)/L) compared to controls. Decreased iron was accompanied by altered expression of genes involved in iron transport and absorption. Based on these findings and the knowledge that Cr(VI), Cr(V), and Cr(IV) can oxidize ferrous iron (Fe^{+2}) to ferric iron (Fe^{+3}) (Buerge and Hug, 1997; Fendorf and Li, 1996), Suh et al. (2014) hypothesized that Cr(VI) may oxidize ferrous (Fe^{+2}) iron to ferric (Fe^{+3}), thereby interfering not only with (Fe^{+2}) absorption in the

intestinal lumen, but also competing with (Fe^{+2}) for heme binding and ferric iron (Fe^{+3}) storage by ferritin in RBCs. Cr(VI), but not Cr(III) (NTP, 2010; Stout et al., 2009), hinders iron aborption in the small intestine, leading to iron deficiency in rats and to a lesser extent in mice. Consistent with this hypothesis, Cr(VI) reduced to Cr(III) has been shown to bind transferrin under physiological conditions (Levina et al., 2016; Deng et al., 2015). Consistent with Suh et al. (2014), Wang et al. (2015) also observed dose-related decreases in iron levels in the liver, kidney, duodenum, and lung in rats exposed to concentrations up to 106.1 mg/L Cr(VI) in drinking water for four weeks; no changes were detected in blood iron levels, but significant decreases in Hgb, MCH, and MCHC levels and increased RBC counts were observed. This evidence that Cr(VI) can inhibit iron absorption suggests that humans with preexisting blood conditions (e.g., anemia, iron deficiency, intestinal bleeding disorders) would be expected to be more sensitive to any potential hematologic effects of Cr(VI) exposure.

Oxidative stress, RBC membrane damage and eryptosis

Both iron deficiency and Cr(VI) exposure have been shown to independently increase oxidative damage. Potassium dichromate, like iron, is a charged heavy metal, and it has been proposed that interaction between iron bound by RBCs alters erythrocyte function and/or formation particularly by targeting the erythron (NTP, 2007, 1997, 1996a, b). Cr(VI) redox results in oxidative damage both to hemoglobin and to the RBC membrane (ATSDR, 2012; NTP, 2007). The increased oxidative damage can initiate pathways leading to erythrocyte injury and eryptosis (i.e., erythrocyte apoptosis) as well as smaller RBCs (Kempe et al., 2006), consistent with observations of decreased MCV in rats and mice (NTP, 2008, 2007).

As discussed in Section 3.2.1, "Respiratory effects other than cancer," evidence of oxidative stress (i.e., increased oxidative 8-OHdG DNA adducts and lipid peroxidation levels, decreased antioxidant levels) has been detected at significant levels in the blood (RBCs, plasma, serum) of workers exposed to Cr(VI) (El Safty et al., 2018; Hu et al., 2018; Xu et al., 2018; Mozafari et al., 2016; Elhosary et al., 2014; Zendehdel et al., 2014; Kalahasthi et al., 2006; De Mattia et al., 2004; Maeng et al., 2004; Wu et al., 2001; Huang et al., 1999; Gromadzińska et al., 1996) (see Appendix Table C-56). In animals, one 4-week drinking water study in male F344 rats exposed to 10.6–106 mg Cr(VI)/L and found increased plasma malondialdehyde (MDA), a reactive marker of lipid peroxidation, and decreased glutathione peroxidase (GSH-Px), an antioxidant enzyme (Wang et al., 2015). Other findings consistent across in vitro studies with primary human RBCs included observation of oxidative stress indicators and eryptosis, including increased MDA levels, changes in antioxidant activity, increased cytosolic Ca^{2+}, increased phosphatidylserine on the outer membrane surface, and decreased ATP (Sawicka and Długosz, 2017; Zhang et al., 2014; Lupescu et al., 2012; Ahmad et al., 2011; Fernandes et al., 1999; Koutras et al., 1964). These effects indicate a loss of membrane integrity, coherent with the microscopic evaluations of blood smears from exposed rats and mice, where evidence of erythrocyte injury, including poikilocytes, erythrocyte fragments/schizocytes,

and keratocytes, were observed after 90 days of Cr(VI) exposure in drinking water (NTP, 2008, 2007). Collectively, the findings of RBC oxidative stress leading to cell membrane damage and eryptosis are a possible pathway leading to the observed changes in RBC size, and are correlative with an erythrogenic response supported by increased RBC counts. However, study durations were limited to ≤90 days and it is not clear if these mechanistic effects would be persistent long-term.

3.2.5.4. *Integration of Evidence*

Overall, the currently available ***evidence suggests*** that Cr(VI) exposure may cause hematologic effects in humans under relevant exposure circumstances. This conclusion is based primarily on *moderate* animal evidence from *high* and *medium* confidence subchronic and chronic studies in rats and mice reporting consistent (across similar exposure durations and doses, sexes, and species), dose-related, and coherent findings (i.e., in RBC, Hgb, MCHC, MCH, and MCV) at 90 days exposure. However, the confidence in these findings was diminished due to the decrease in magnitude of the collective effect by 12 months, with many findings returning to normal or near normal levels (generally, with a magnitude of change <10% compared to controls). Given the absence of correlative findings of apparent RBC injury from blood smears (other than smaller RBCs that were hypochromic) and the absence of supportive mechanistic findings (such as iron deficiency and oxidative stress) at 12 months, there exists a large amount of uncertainty regarding the adverse versus adaptive nature of the observed effects at exposure durations greater than 90 days. In particular, the biological significance of the response at 12 months is uncertain; comparing the observed decreases in Cr(VI)-exposed rats to reference ranges ("historical controls") indicates that the observed findings fall within the low-normal or just below the low normal range of results typically observed for the tested strains.

Although the adversity or clinical relevance of the observed changes in any one of the individual hematologic parameters in isolation is unclear, and there is a large degree of uncertatinty in the adversity of the effect at 12 months, the interpretation of the collective animal evidence still signals a potential concern, particularly when considering that similar findings in humans would likely warrant follow-up testing and evaluation by a hematologist. Supporting evidence of Cr(VI)-induced iron deficiency and oxidative stress indicates potential pathways leading to the observed findings of hypochromic microcytic anemia, consistent with the microscopic evaluation of blood smears (with findings of damage to the erythron), strengthens the evidence for an effect at 90 days. Information including iron levels and ferritin tests that are useful for evaluating the amount of stored iron were not available at exposure durations >90 days, making it difficult to confirm whether the diminished effects at 12 months could still be considered adverse. Therefore, although there remains a (weaker) signal for an effect at 12 months, there exists a large amount of uncertainty as to the adversity of the effect. Integrated evidence for the hematologic effects of Cr(VI) exposure from human, animal, and mechanistic studies is summarized in an evidence profile table (Table 3-31). The only available human study examining potential hematologic effects was considered *uninformative*; therefore, there is *indeterminate* human evidence of hematologic effects.

However, the mechanistic evidence suggests that humans with preexisting blood conditions (e.g., anemia, iron deficiency, intestinal bleeding disorders) would be expected to be more sensitive to any potential hematologic effects of Cr(VI) exposure.

Table 3-31. Evidence profile table for hematologic effects

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from studies of exposed humans					⊕⊕⊙ The **evidence suggests** that Cr(VI) may cause hematologic effects in humans. *Primary basis:* One *high* confidence subchronic and one *high* confidence chronic study with consistent findings across species and dose duration with coherent effects on RBC indices suggesting microcytic anemia, and supportive mechanistic findings of of Cr(VI)-induced iron deficiency and RBC damage. However, the confidence in these findings is reduced by the uncertainty regarding the adverse versus adaptive nature of the observed effects, particularly given the near amelioration of effects after one year, precluding a higher confidence judgment (i.e., *evidence indicates*).
HEMATOLOGIC PARAMETERS Four *low* confidence studies, two in occupationally exposed adult workers and two in general population adults	Exposure to Cr(VI) was associated with lower hemoglobin and/or hematocrit in one study ($p < 0.05$), while two studies reported in the opposite direction (higher hemoglobin and hematocrit in one study, higher red blood cells in one), and one study reported no association.	No factors noted	• *Low* confidence studies • Unexplained inconsistency across studies	⊙⊙⊙ *Indeterminate* The available evidence is inconsistent across *low* confidence studies.	
Evidence from animal studies					
Hematology Six *high* confidence studies in adult rats and mice • 28-day oral • 9-week oral (2 studies) • Continuous breeding oral • 90-day oral • 2-year oral Five *medium* studies in adult male and female rats	Hematologic effects included consistent decreases in Hgb, MCV, MCH, and MCHC, and increased RBC counts and reticulocytes at 90 days; marginal (near low-normal) decreases in MCV, MCH and increase in RBC at 12 months. Most findings returned to near normal by 12 month exposures. 90 day findings were coherent with microscopic findings of RBC damage including smaller size and hypochromic appearance	• *Consistent* findings of decreased Hgb, MCH, MCHC, MCV, and increased RBC across species and sexes in subchronic and chronic studies • *Coherence* of decreased Hgb, MCH, MCHC, and MCV with increased RBC and reticulocytes	• Lack of duration-dependence (effects of Cr(VI) decreased with longer-term exposures) • Uncertainty of the biological significance of effects at 12 months	⊕⊕⊙ *Moderate* *High* confidence subchronic and chronic studies with consistent findings across species (although rats are more sensitive) and sexes based on coherent effects across multiple related endpoints (RBC, Hgb, MCHC, MCH and	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
• 28-day oral (3 studies) • 30-day oral • 90-day inhalation Four *low* confidence studies in male rats and mice • 28- and 90-day inhalation (2 experiments, 1 study) • 30- and 90-day inhalation (2 experiments, 1 study) • 18-month inhalation • Short-term oral study during pregnancy	that were consistent with Cr(VI)-induced iron deficiency.	• Dose-response gradient for RBC, MCH, MCV, MCHC, Hgb (rat, 90-day) • Mechanistic evidence of iron deficiency and altered pathways involved in iron metabolism in rats exposed for ≤90 days provides biological plausibility		MCV). Strong dose response relationship (primarily at 90 day for RBC, Hgb, MCV, MCHC, MCH in rats). Some uncertainty around biological relevance of the effect as the magnitude of the change compared to controls decreased by 12 mo. Strong mechanistic support for anemia (indicated by decreased Hgb, MCV, MCHC, MCH) provided by mechanistic studies demonstrating Cr(VI) induced iron deficiency and oxidative damage in the blood of exposed humans and animals, and regenerative responses consistent with smaller RBC size.	*Human relevance:* Human evidence was primarily inconsistent and *low* confidence. Without evidence to the contrary, effects in rats and mice are considered relevant to humans. *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible populations and life stages*: People with preexisting blood conditions (e.g., anemia, iron deficiency, chronic intestinal bleeding disorders) are expected to be susceptible populations.
Mechanistic Evidence					
Biological events or pathways	**Summary of key findings and interpretation**			**Judgments and rationale**	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Oxidative Stress	*Interpretation:* Oxidative stress caused by Cr(VI) reactive intermediates may lead to erythrocyte lipid peroxidation, membrane damage, and eryptosis. *Key Findings:* • Consistent evidence of oxidative stress in the blood of workers exposed to Cr(VI) (see Section 3.2.1, "Respiratory effects other than cancer") • Increased oxidative stress levels in plasma in one in vivo study of rats exposed in drinking water for 4 weeks • Cr(VI) increased markers of oxidative stress, cellular injury and death in primary human RBCs in vitro, including MDA, decreased antioxidant enzymes, increased cytosolic Ca^{2+}, membrane destabilization, and decreased ATP			Biologically plausible pathways leading to the observed clinical pathology and microscopic evaluation of blood smears that included Cr(VI) oxidation of ferrous to ferric iron, potentially altering bioavailability, oxidative damage to the RBC leading to increased turnover and smaller size, and Cr(VI) interference with iron metabolism leading to iron deficiency. Support for oxidative stress occurring in the blood of humans is provided by consistent findings of increased markers of oxidative stress in exposed workers.	
Iron Deficiency	*Interpretation:* Interference with iron homeostasis due to interactions with Hgb, iron and its transporter proteins may also contribute to hematologic toxicity. *Key Findings:* • Cr(VI) interaction with iron may alter RBC binding and erythrocyte function or formation • Cr(VI) reduced to Cr(III) may bind transferrin, an iron transporter, under physiological conditions • Additional in vivo evidence suggests Cr(VI)-induced alterations in iron homeostasis including dose-dependent decreases in total iron in various tissues, altered gene regulation, and increased ratios of RBC Cr(VI):plasma Cr(VI)				

3.2.6. Immune effects

The purpose of the immune system is to provide protection from infections and, in some cases, the development of neoplasms. A properly functioning immune system involves a delicate interplay among many cell types working in concert to properly regulate the immune response. The immune system is integrated into tissues, organs and peripheral sites throughout the body. For this reason, xenobiotic exposure by virtually any route can adversely impact components of the immune system. Modulation of the immune system in either direction can result in dysfunction. Xenobiotic exposure can alter primary immune sites important for immune cell maturation, including the bone marrow, liver, thymus, and Peyer's patches. Secondary lymphoid sites (i.e., spleen, lymph nodes, tonsils) can also be impacted by exposure to immunotoxicants. Immunotoxicity may be expressed as immunosuppression, unintended stimulation of immune responses, hypersensitivity, or autoimmunity (IPCS, 2012). Data from functional assays provide the most sensitive and specific evidence of immune hazard.

This synthesis is organized and summarized based on the World Health Organization's *Guidance for Immunotoxicity Risk Assessment for Chemicals* (IPCS, 2012) that describes best approaches for weighing immunotoxicological data. Within this framework, data from endpoints observed in the absence of an immune stimulus (e.g., levels of serum immunoglobulins, white blood cell (WBC) counts, WBC differentials, T cell subpopulations, immune organ weights) are not sufficient on their own to draw a conclusion regarding immune hazard but may provide useful supporting evidence, especially when evaluated in the broader context of functional data (IPCS, 2012). Consequently, the sections that follow are organized into two categories: the more informative measures of immune system function and supporting immune system data.

3.2.6.1. *Human Evidence*

Study evaluation summary

Table 3-32 summarizes the human epidemiology studies considered in the evaluation of the potential effects of Cr(VI) on the immune system. These consist of nine cross-sectional occupational studies conducted among workers in four industries with known risk of exposure to Cr(VI) in a range of geographical locations. They include two studies of chrome-plating workers (Kuo and Wu, 2002; Verschoor et al., 1988), two studies of tannery workers (Mignini et al., 2009; Mignini et al., 2004), two studies of chemical plant workers (Qian et al., 2013; Tanigawa et al., 1998), one study of chromate production workers (Wang et al., 2012a) and one study of plastic workers (Boscolo et al., 1997). In addition, one cross-sectional study assessed the effects of Cr(VI) exposure on the general population in Greece (Sazakli et al., 2014). All studies were considered *low* confidence. Four additional studies were identified and classified as *uninformative* due to critical deficiencies in exposure methods sensitivity and/or confounding and were not considered further (Islam et al., 2019; Khan et al., 2013; Katiyar et al., 2008; Snyder et al., 1996). Information on study evaluation

are provided in the text below and in Table 3-32. Available evidence in human studies was limited to *ex vivo* WBC function, white blood cells (number, type, and T cell subpopulations), immunoglobulin levels, complement levels, and cytokine levels.

While cytokines are critical for maintaining immune homeostasis, cytokine data, especially measures of blood cytokines, can be challenging to interpret as primary evidence of immune hazard (Tarrant, 2010). Changes in cytokine levels can be associated with many different types of tissues and toxicities, as part of cell differentiation to different immune cell types, or including site-specific inflammation, which reflects an immune response to tissue injury but not necessarily an impact on or impairment of immune function. For this reason, cytokine secretion data (in the absence of a stimulus) were not considered apical outcomes for the purpose of identifying immune hazard, but rather as supporting evidence for understanding mechanisms of immune disruption and are summarized in the Mechanistic and Supporting Evidence section below without systematic review.

Allergic sensitization can occur in some individuals exposed to Cr(VI) (OSHA, 2006). Because the primary exposure route (i.e., dermal) is outside the scope defined by the PECO criteria, evidence for allergic hypersensitivity responses following Cr(VI) exposure has not been comprehensively reviewed, but is briefly summarized in the Mechanistic and Supporting Evidence section below if the exposures or outcomes were relevant to non-dermal Cr(VI) exposures

Table 3-32. Summary of human studies for Cr(VI) immune effects and overall confidence classification [high (H), medium (M), low (L)] by outcome. Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Exposure Measurement	Study Design	*Ex vivo* white blood cell function[a]	White blood cells (hematology)	White blood cells (subpopulations)	Immunoglobulin levels
Boscolo et al. (1997)	Plastic workers exposed to lead chromate	Italy	Air	Cross-sectional	–	L	L	L
Kuo and Wu (2002)	Chrome-plating workers	Taiwan	Urine, air	Cross-sectional	–	–	L	–
Mignini et al. (2004)	Tannery workers	Italy	Dust, blood, urine	Cross-sectional	L	–	L	–
Mignini et al. (2009)	Tannery workers	Italy	Air, blood, urine	Cross-sectional	L		L	–
Qian et al. (2013)	Chemical plant workers	China	Work categories, validated by air,	Cross-sectional	–	–	–	L

Author (year)	Industry	Location	Exposure Measurement	Study Design	*Ex vivo* white blood cell function[a]	White blood cells (hematology)	White blood cells (subpopulations)	Immunoglobulin levels
			urine, blood samples					
Sazakli et al. (2014)	General population	Greece	Urine, Hair, Modeled lifetime chromium exposure dose	Cross-sectional	–	L	–	–
Tanigawa et al. (1998)	Chemical plant workers	Japan	Work category	Cross-sectional	–	–	L	–
Verschoor et al. (1988)	Chrome platers, stainless-steel welders	Netherlands	Work categories, validated by urine samples	Cross-sectional	–	–	–	L
Wang et al. (2012a)	Chromate production workers	China	Urine	Cross-sectional	–	L	–	–

[a]*Ex vivo* white blood cell function is more informative of immune system function, while the other measures provide supporting immune system data.

Synthesis of Human Evidence

More informative measures of immune system function

Ex vivo WBC function Ex vivo WBC functional assays (e.g., NK cell activity, phagocytosis, proliferative responses) are performed outside the body using isolated cells collected from exposed individuals. These assays are considered clear evidence of adverse immunosuppression (IPCS, 2012). Two studies examined the association between occupational Cr(VI) exposure and *ex vivo* WBC function (Table 3-32). Both studies of tannery workers were *low* confidence, with *deficient* ratings in participant selection, exposure measurement and sensitivity (Mignini et al., 2009; Mignini et al., 2004). Mignini et al. (2009) did not observe an effect on phagocytosis by PMNs or NK cell activity, but effects of Cr(VI) exposure on the mitogen-induced proliferative response was consistently observed in both studies (Mignini et al., 2009; Mignini et al., 2004) (Table 3-33). Compared to controls, lymphocytes harvested from the exposed workers were stimulated to proliferate to a greater extent in the presence of the T cell mitogen phytohemagglutinin (PHA) (Mignini et al., 2009). The T cell mitogen concanavalin A (ConA) also stimulated lymphocytes collected from these workers (Mignini et al., 2009; Mignini et al., 2004), and there was evidence that the effect of Cr(VI) exposure on ConA stimulation may be affected by HLA haplotype (Mignini et al., 2004). The B cell mitogen, lipopolysaccharide (LPS), had no effect on lymphocyte proliferation following Cr(VI) exposure (Mignini et al., 2009).

Table 3-33. Associations between Cr(VI) exposure and *ex vivo* WBC function in epidemiology studies

Reference, confidence	Population	Exposure measure	Exposure levels	Comparison and effect estimate	Endpoint
Mignini et al. (2004), *low*	Cross-sectional study in Italy of 20 exposed and 24 unexposed workers	Cr levels in blood and urine	NR	ANOVA and the Student's t test	Sig. inc. in mitogen-stimulated lymphocyte proliferation (ConA) in exposed groups (pooled data from both exposure groups)
					Sig. inc. in mitogen-stimulated lymphocyte proliferation in exposed HLA-B8-DR3-negative group to ConA, but not in the HLA-B8-DR3-positive group (pooled data from both exposure groups)
Mignini et al. (2009), *low*	Cross-sectional study in Italy of 40 exposed tannery workers and 44 unexposed workers	Cr levels in urine, 3 categories	~0.6, 0.4, 0.2 ug/L	Means by exposure category (not reported)	Sig. inc. in mitogen-stimulated lymphocyte proliferation in high exposure group to PHA and ConA, but not to LPS
					No effect on percent phagocytosis, phagocytosis index, or killing percent by PMNs
					No effect on NK cell activity, data not shown

Supportive immune system data

Immunoglobulin levels

Three studies examined the association between Cr(VI) exposure and nonspecific immunoglobulin levels (Table 3-32). All three studies were *low* confidence and had *deficient* ratings in participant selection, outcome ascertainment, and confounding (Qian et al., 2013; Boscolo et al., 1997; Verschoor et al., 1988). Immunoglobulin levels are difficult to interpret alone without a controlled immune challenge preceding the measurement. Among these studies (Table 3-34), exposed workers had lower levels of IgA and IgG Qian et al. (2013), but levels were unaffected in Boscolo et al. (1997). Levels of IgG were also unaffected in Verschoor et al. (1988). Serum levels of IgM were unaffected by Cr(VI) exposure in the only two studies that investigated this isotype (Qian et al., 2013). IgE levels were unaffected in the only study that investigated this isotype (Boscolo et al., 1997).

Table 3-34. Associations between Cr(VI) exposure and immunoglobulin (Ig) levels in epidemiology studies

Reference, confidence	Population	Exposure measure	Exposure levels	Comparison and effect estimate	IgG	IgM	IgA	IgE
Boscolo et al. (1997), *low*	Cross-sectional study in Italy of 15 plastic workers exposed to lead chromate and 15 unexposed workers from the same area	Exposed/ unexposed. Chromium levels measured in blood and urine. Levels in exposed were significantly higher in urine, but similar to unexposed in blood	Chromate ranged in air from 0.1 to 5.7 µg/m^3	Median in mg/dl (25th–75th) for exposed and unexposed	Exposed: 1240 (991–1296) Unexposed: 1151 (942–1276)	Exposed: 118 (75–140) Unexposed: 79 (58–111)	Exposed: 193 (182–282) Unexposed: 277 (186–292)	NA
Verschoor et al. (1988), *low*	Cross-sectional study in the Netherlands of 21 chrome platers, 38 SS welders, 16 boilermakers, and 63 unexposed workers	Work categories, validated by urine samples	9, 3, 1, 0.4 µg/g creatinine in urine	Mean ± SD	Chrome platers: 11.6 ± 3.2 SS welders: 11.1 ± 2.6 Boilermakers: 11.1 ± 2.8 Controls: 11.6 ± 2.4	NA	NA	NA
Qian et al. (2013), *low*	Cross-sectional study in China of 56 workers exposed to potassium dichromate and 50 unexposed individuals living 20 km from factory	Exposed/ unexposed validated by air sampling	14.4 ± 18.1 µg/m^3	Except for IgE, mean in g/L ± SD for exposed and unexposed	Exposed: 10.9 ± 2.5 Unexposed: 12.4 ±2.1 p = 0.03*	Exposed: 1.2 ± 0.5 Unexposed: 1.0 ± 0.4	Exposed: 2.4 ± 0.9 Unexposed: 2.8 ± 1.2 p = 0.04*	Exposed (Median g/L (quartile)] 55.2 (157.4) Unexposed 81.9 (237.1)

NA = not applicable.

WBC counts (hematology)

For WBC measures, three studies were available (Table 3-32). All three studies were cross-sectional studies of Cr(VI) exposure and measured WBC counts, or related measures,

including counts of total WBCs, lymphocytes and granulocytes (Table 3-35). All studies were *low* confidence. Sazakli et al. (2014) was *deficient* only for exposure measurement, while the remaining studies were *deficient* for multiple domains, including participant selection (Wang et al., 2012a; Boscolo et al., 1997), confounding (Wang et al., 2012a; Boscolo et al., 1997), and outcome ascertainment (Boscolo et al., 1997). Among these studies, one reported an increase in WBCs with higher exposure to Cr(VI). This was statistically significant for total WBCs, and non-statistically significant increases were also observed for lymphocytes and neutrophils (Wang et al., 2012a). Two other studies indicated no increase (Sazakli et al., 2014; Boscolo et al., 1997), with one indicating non-statistically significant decreases for lymphocytes and WBCs(Boscolo et al., 1997) (Table 3-35).

Table 3-35. Associations between Cr(VI) exposure and WBC counts in epidemiology studies

Reference, confidence	Population	Exposure measure	Exposure levels	Comparison and effect estimate	Total WBC (count 10^9/L)	Lymphocytes (count 10^9/L)	Granulocytes (count 10^9/L)	Neutrophils (count 10^9/L)
Sazakli et al. (2014) *low*	Cross-sectional in Greece, general population; Two exposure groups (n = 237) and controls (n = 67)	Chromium levels measured in blood and hair. Estimated lifetime chromium exposure dose calculated using concentration in drinking water, intake rate, and body weight	NR	Regression coefficients for calculated lifetime exposure dose and Cr in hair	Lifetime dose: −0.03 Hair: 0.07	Lifetime dose: 0.02 Hair: 0.1	Lifetime dose: −0.01 Hair: 0.03	NA
Boscolo et al. (1997), *low*	Cross-sectional study in Italy of 15 plastic workers exposed to lead chromate and 15 unexposed workers from the same area	Exposed/ unexposed. Chromium levels measured in blood and urine. Levels in exposed were significantly higher in urine, but similar to unexposed in blood	Chromate ranged in air from 0.1 to 5.7 µg/m^3	Median (25th–75th) for exposed and unexposed	Exposed: 6764 (5940–7180) Unexposed: 6776 (5680–8190)	Exposed: 2340 (1490–2915) Unexposed: 2730 (2300–3090)	NA	NA

Reference, confidence	Population	Exposure measure	Exposure levels	Comparison and effect estimate	Total WBC (count 10^9/L)	Lymphocytes (count 10^9/L)	Granulocytes (count 10^9/L)	Neutrophils (count 10^9/L)
Wang et al. (2012a), *low*	Cross-sectional study in China of 86 chromate production workers and 45 unexposed workers	Exposed/ unexposed. Chromium levels measured in urine were significantly higher in exposed workers	<50 µg/m^3	Mean (SD) for exposed and unexposed	Exposed: 7.0 (1.7) Unexposed: 6.2 (1.3) *p* = 0.03* Mixed WBC[a] Exposed: 0.6 (0.3) Unexposed: 0.4 (0.1)	Exposed: 2.2 (0.7) Unexposed: 2.1 (0.5)	NA	Neutrophils Exposed: 4.1 (1.4) Unexposed: 3.7 (1.0)

NA = not applicable.

[a] Cell mixture containing neutrophils, eosinophils, basophils and mast cells.

Lymphocyte subpopulations

Five studies examined the association between Cr(VI) exposure and lymphocyte subpopulations (Table 3-32). All five studies were *low* confidence cross-sectional studies of Cr(VI) exposure and white blood cell counts (Qian et al., 2013; Mignini et al., 2009; Mignini et al., 2004; Tanigawa et al., 1998; Boscolo et al., 1997). Three studies reported decreased CD4+, CD8+, and CD3+ cells with higher exposure to Cr(VI) (Kuo and Wu, 2002; Tanigawa et al., 1998; Boscolo et al., 1997), with statistical significance in two. Two studies reported no qualitative effects on the levels of CD3+, CD4+, CD8+, DC19 (Mignini et al., 2009; Mignini et al., 2004), CD56 (Mignini et al., 2004), CD16+/CD56+ and CD4/CD8 (Mignini et al., 2009) levels (Table 3-36).

Table 3-36. Associations between Cr(VI) exposure and lymphocyte subpopulations in epidemiology studies

Reference, confidence	Population	Exposure measure	Exposure levels	Comparison and effect estimate	CD4+	CD8+	CD3+	CD19	CD56
Boscolo et al. (1997), *low*	Cross-sectional study in Italy of 15 plastic workers exposed to lead chromate and 15 unexposed workers from the same area	Exposed/ unexposed. Chromium levels measured in blood and urine. Levels in exposed were significantly higher in urine, but similar to unexposed in blood	Chromate ranged in air from 0.1 to 5.7 µg/m^3	Median (25th–75th) for exposed and unexposed	Exposed: 870 (585–1135) Unexposed: 1140 (970–1240) $p < 0.05$*	Exposed: 710 (435–795) Unexposed: 810 (570–870)	Exposed: 1630 (1035–1995) Unexposed: 1890 (1680–2170)	Exposed: 180 (150–280) Unexposed: 330 (260–460)	NA
Tanigawa et al. (1998), *low*	Cross-sectional study in Japan of 19 retired chromate workers and 13 unexposed workers	Exposed/unexposed. No validation of exposure levels.	NR	Mean ± SD for exposed and unexposed, by smoking status	Exposed smokers: 790 ± 260 Exposed nonsmokers: 870 ± 510 Unexposed smokers: 1660 ± 570 Unexposed non-smokers: 1250 ± 450 $p < 0.05$*	Exposed smokers: 470 ± 250 Exposed nonsmokers: 330 ± 200 Unexposed smokers: 540 ± 280 Unexposed nonsmokers: 670 ± 480 $p < 0.05$*	Exposed smokers: 1140 ± 380 Exposed nonsmokers: 1150 ±640 Unexposed smokers: 2110 ± 530 Unexposed nonsmokers: 1840 ± 650 $p < 0.05$*	NA	NA
Kuo and Wu (2002), *low*	Cross-sectional study in Taiwan of 27 workers from 5 Cr	Chromium levels in air samples and urine.	NR	Beta (SE) for moderate and high urine Cr vs. low group	Moderate: −0.03 (2.5) High: −0.2 (4.0)	Moderate: −1.8 (2.3) High: −6.5 (3.6)	NA	NA	NA

Reference, confidence	Population	Exposure measure	Exposure levels	Comparison and effect estimate	CD4+	CD8+	CD3+	CD19	CD56
	electroplating plants and 19 unexposed workers			Correlation coefficient with airborne Cr	−0.06	−0.08	NA	NA	NA
Mignini et al. (2004), *low*	Occupational exposure study in Italy of 20 exposed and 24 unexposed workers	Cr levels in blood and urine	NR	ANOVA and the Student's t test	No changes reported, data not shown	No changes reported, data not shown	No changes reported, data not shown	No changes reported, data not shown	No changes reported, data not shown
Mignini et al. (2009), *low*	Cross-sectional study in Italy of 40 exposed tannery workers and 44 unexposed workers	Cr levels in urine, 3 categories	~0.6, 0.4, 0.2 ug/L	Mean ± SD for exposed and unexposed, Duncan Multiple Range,' 'Newman-Keuls, Mann-Whitney test	No changes reported, data not shown	No changes reported, data not shown	No changes reported, data not shown	No changes reported, data not shown	No changes reported, data not shown

NA = not applicable.

3.2.6.2. *Animal Evidence*

This section focuses on outcomes considered informative for the identification of chemical-induced adverse effects on the immune system (IPCS, 2012; U.S. EPA, 1998b), particularly changes in response to an immune challenge, including effects on antibody responses, host resistance, and *ex vivo* white blood cell (WBC) function. Supporting data collected from animals in the absence of an immune challenge were also considered, including effects on immune organ pathology, nonspecific immunoglobulin levels, immune organ weights, WBC counts (spleen, thymus, bone marrow and hematology), and lymphocyte subpopulations. In addition to the evidence syntheses below, the study findings have been summarized in Appendix Table C-42.

Study evaluation summary

Table 3-37 summarizes the animal toxicology studies considered in the evaluation of the effects of Cr(VI) on the immune system. These studies consist of one oral diet (NTP, 1996a), one oral gavage (Krim et al., 2013), 11 drinking water (Karaulov et al., 2019; Jin et al., 2016; Wang et al., 2015; NTP, 2008, 2007, 2006a, b, 2005; Shrivastava et al., 2005a; Shrivastava et al., 2005b; Snyder and Valle, 1991), and eight inhalation studies (Cohen et al., 2010; Cohen et al., 2006; Kim et al., 2004; Cohen et al., 1998; Glaser et al., 1990; Glaser et al., 1986; Johansson et al., 1986b; Glaser et al., 1985). These studies used a variety of mouse and rat strains, including BALB/c, B6C3F1, *am3*-C57BL/6, and Swiss mice (NTP, 2008, 2007, 2005; Shrivastava et al., 2005a; Shrivastava et al., 2005b; NTP, 1996a) and Sprague-Dawley, F344, F344/N, Wistar, and albino Wistar rats (Karaulov et al., 2019; Wang et al., 2015; Krim et al., 2013; Cohen et al., 2010; NTP, 2008, 2007; Cohen et al., 2006; NTP, 2006a, b; Kim et al., 2004; Cohen et al., 1998; Snyder and Valle, 1991; Glaser et al., 1990; Glaser et al., 1986; Glaser et al., 1985).

Table 3-37. Summary of included studies for Cr(VI) immunological effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive data graphic for rating rationales.

				More informative measures[b]			Supporting evidence				
Author (year)	Species (strain)	Exposure design	Exposure route	Host resistance	Antibody responses	Ex vivo WBC function	Immune organ pathology	Immunoglobulin levels	Immune organ weights	WBC counts and differentials (spleen, thymus, bone marrow)	WBC counts (hematology)
NTP (2005)	Mouse (B6C3F1)	Short-term	Drinking water	-	H	H	H	H	H	H	M
NTP (2006b)	Rat (Sprague-Dawley)	Short-term	Drinking water	-	H	H	M	H	H	H	M
NTP (2006a)	Rat (F344)	Short-term	Drinking water	-	H	H	M	H	H	H	M
NTP (2007)	Rat (F344/N); Mice (B6C3F1, BALB/c, *am3*-C57BL/6)	Subchronic	Drinking water	-	-	-	H	-	H	-	H
NTP (2008)	Rat (F344/N); Mice (B6C3F1)	Chronic	Drinking water	-	-	-	H	-	-	-	H
Karaulov et al. (2019)	Rat (Wistar)	Chronic	Drinking water	-	-	M	L	-	M	M	-
Wang et al. (2015)	Rat (Sprague-Dawley)	Short-term	Drinking water	-	-	-	-	-	-	-	M
Jin et al. (2016)	Mouse (ICR)	Short-term	Drinking water	-	-	-	-	-	M	-	-
Shrivastava et al. (2005a)	Mouse (Swiss)	Short-term & subchronic	Drinking water	-	-	-	-	-	-	-	L
Shrivastava et al. (2005b)	Mouse (Swiss)	Short-term & subchronic	Drinking water	-	-	L	-	-	L	-	-
Snyder and Valle (1991)	Rat (F344)	Short-term	Drinking water	-	-	L	-	-	-	-	-
Krim et al. (2013)	Rat (albino Wistar)	Short-term	Gavage	-	-	-	-	-	-	-	M

				More informative measures[b]			Supporting evidence				
Author (year)	**Species (strain)**	**Exposure design**	**Exposure route**	**Host resistance**	**Antibody responses**	**Ex vivo WBC function**	**Immune organ pathology**	**Immunoglobulin levels**	**Immune organ weights**	**WBC counts and differentials (spleen, thymus, bone marrow)**	**WBC counts (hematology)**
NTP (1996a)	Mouse (BALBC)	Subchronic	Diet	-	-	-	H	-	-	-	H
Cohen et al. (1998)	Rat (F-344)	Short-term	Inhalation	-	-	M	-	-	-	-	-
Cohen et al. (2006)	Rat (F-344)	Short-term	Inhalation	M	-	-	-	-	-	-	-
Cohen et al. (2010)	Rat (F-344)	Short-term	Inhalation	M	-	-	-	-	-	-	-
Glaser et al. (1985)	Rat (Wistar)	Short-term & subchronic	Inhalation	-	L	L	-	M	M	-	L
Glaser et al. (1986)	Rat (Wistar)	Chronic	Inhalation	-	-	-	M	L	L	-	M
Glaser et al. (1990)	Rat (Wistar)	Short-term & subchronic	Inhalation	-	-	-	-	L	-	-	M
Kim et al. (2004)	Rat (Sprague-Dawley)	Subchronic	Inhalation	-	-	-	-	-	M	-	M
Johansson et al. (1986b)	Rabbit (strain not specified)	Chronic	Inhalation	-	-	M	-	-	-	-	-

[a]In addition to these included studies, there were three animal toxicology studies reporting immunotoxicity outcomes that met PECO criteria but were found to be *uninformative* at the study evaluation stage for reporting or attrition Geetha et al. (2003), outcomes measures Nettesheim et al. (1971), and outcomes measures, exposure methods, reporting or attrition, confounding variable control, and selection or performance Kumar and Barthwal (1991).

[b]Host resistance, antibody responses, and *ex vivo* WBC function are more informative as measures of immune system function. The remaining measures provide supporting immune system data.

Synthesis of Animal Evidence

More informative measures of immune system function

Host resistance

Host resistance assays are considered the gold standard of immunotoxicity testing because clearance of a self-replicating infectious agent or neoplastic disease requires the integration of

immune system responses to protect the host, and disruption of this integrated response at any point can be detected as a reduction in host resistance. The effect of exposure to Cr(VI) (0.119 mg/m^3 for 5 h/d for 5 consecutive days) on *in situ* clearance of pneumonia-inducing *Listeria monocytogenes* (24, 48 and 72 h timepoints) was investigated in two *medium* confidence studies of male F344 rats (Cohen et al., 2010; Cohen et al., 2006). Compared to the air-exposed control, pathogen clearance was reduced in rats exposed to high soluble (Na_2CrO_4) and low soluble ($CaCrO_4$) Cr(VI), but only when measured at the 72 h timepoint (Cohen et al., 2010; Cohen et al., 2006). The authors noted that the reduction in pathogen clearance did not correlate with lung chromium burden (Cohen et al., 2010; Cohen et al., 2006). Overall, available data suggest that short-term exposure to chromium may reduce *in situ* bacterial clearance in the lung (i.e., phagocyte recruitment and bacterial lysis). Since the model used in these studies is a targeted host resistance model designed to evaluate local pathogen clearance by macrophages, future studies using a comprehensive host resistance model (e.g., influenza virus) would be useful for developing a better understanding of the potential for Cr(VI) exposure to impair host resistance.

Antibody responses

Cr(VI) exposure increased IgM antibody-forming cell responses to sheep red blood cells in three *high* confidence 28-day NTP studies (NTP, 2006a, b, 2005), but the effect was only significant in two of the studies (NTP, 2006a, 2005) and the same effect was not observed in a repeat assay performed by NTP (2005). One 90-day inhalation study, found to be *low* confidence due to deficiencies in the presentation of results, also reported increased IgM antibody-forming cell responses to sheep red blood cells (Glaser et al., 1985). These investigations were performed in female B6C3F1 mice and two different strains of female rat exposed to a broad and overlapping range of Cr(VI) in drinking water (5−180 mg/L) and according to experimental protocols sufficient for the detection of alterations in antibody cell forming responses.

Antibody response studies only provide information on the number of antibody producing plasma cells at the time of assay completion, but these studies do not provide any information on the levels of antigen-specific antibodies in the serum of Cr(VI)-exposed animals. Three *high* confidence NTP studies in mice and rats exposed to Cr(VI) in drinking water for 28 days showed no effect on serum titers of total IgM antibodies specific for two different T cell-dependent antigens (NTP, 2006a, b, 2005).

Overall, Cr(VI) exposure increased antibody responses to sheep red blood cells but did not alter the serum antibody titer following exposure to Cr(VI).

Ex vivo WBC function

In a *low* confidence study by Glaser et al. (1985), phagocytic activity was significantly increased compared to the control group in alveolar lung macrophages isolated from male Wistar rats exposed to Cr(VI) (up to 0.050 mg/m^3) as sodium dichromate by inhalation for 28 and 90 days but was decreased significantly following a 90-day exposure to 0.20 mg/m^3. Findings by the companion study (Glaser et al., 1990) also showed changes characteristic of acute lung injury and

inflammatory lung responses (see Section 3.2.1.2). In a second, *medium* confidence inhalation exposure study, phagocytosis by rabbit alveolar macrophages was unaffected following exposure to 0.9 ± 0.4 mg/m^3 Cr(VI) as sodium chromate for 4–6 weeks (Johansson et al., 1986b). The absence of an effect in Johansson et al. (1986b) may have been due to a 3-day gap between cessation of exposure to Cr(VI) and evaluation of phagocytic activity. In Glaser et al. (1985), the clearance of inhaled iron oxide was lower in the lungs of rats exposed to 0.20 mg/m^3 Cr(VI) for 42 days, though the number of lung macrophages was also reduced relative to the control group. Consequently, the observed decrease in lung clearance cannot be attributed definitively to a defect in phagocytosis. In a third *low* confidence study, however, phagocytic activity of mouse splenic macrophages was reduced from 92% in control male Swiss mice to 36% in mice exposed to 14.8 mg/kg-day Cr(VI) in drinking water for 9 weeks (Shrivastava et al., 2005b).

Cr(VI) exposure had no effect on natural killer (NK) cell activity, mixed lymphocyte response (MLR), and anti-CD3 stimulation of lymphocytes in three *high* confidence drinking water studies (NTP, 2006a, b, 2005) and one *low* confidence drinking water study (Snyder and Valle, 1991). The studies were performed in female B6C3F1 mice and two different strains of female rats (Sprague-Dawley and F344) exposed to a broad and overlapping range of Cr(VI) in drinking water (5–180 mg/L) and according to experimental protocols sufficient for the detection of alterations in cell-mediated responses.

Mitogen-induced proliferative response was consistent in three *low* confidence studies (Shrivastava et al., 2005b; Snyder and Valle, 1991; Glaser et al., 1985). Spleen cells isolated from male Swiss mice exposed to Cr(VI) in drinking water (14.8 mg/kg-day) for 9 weeks were stimulated to proliferate with ConA, but the investigators did not conduct statistical analyses of the findings (Shrivastava et al., 2005b). Increased proliferation was observed in splenocytes isolated from F344 rats exposed to Cr(VI) in drinking water (100 or 200 mg/L) for 3 weeks when stimulated with the T lymphocyte mitogen ConA or B lymphocyte mitogen lipopolysaccharide (LPS) (Snyder and Valle, 1991). Spleen cells isolated from rats exposed to Cr(VI) by inhalation (0.20 mg/m^3) for 90 days were stimulated to proliferate to a greater extent than controls by ConA (Glaser et al., 1985).

Mitogen-induced cytokine secretion was evaluated in two *medium* confidence studies (Karaulov et al., 2019; Cohen et al., 1998). Spleen cells isolated from rats exposed to Cr(VI) in drinking water for 45, 90, and 135 days and stimulated with ConA secreted less IL-6 (day 135) and more IL-4 (day 45, 90, and 135) than controls, while secretion of IL-10 and IFNγ were unaffected by treatment (Karaulov et al., 2019). Compared to control, secretion of IL-1 and TNFα were decreased in pulmonary alveolar macrophages harvested from rats exposed to Cr(VI) by inhalation for 4 weeks and stimulated with LPS whereas a nonsignificant increase in IL-6 secretion was observed (Cohen et al., 1998).

Compared to the control group, exposure to Cr(VI) (0.36 mg/m^3) by inhalation for 28 days had no effect on spontaneous O_2- and H_2O_2 production in the presence or absence of IFN-γ at 4 weeks, but increased opsonized zymosan-stimulated O_2-, and decreased H_2O_2 production

stimulated by opsonized zymosan in the presence of IFN-γ (Cohen et al., 1998). Cr(VI) had no effect on LPS-stimulated nitric oxide (NO) production at 4 weeks but reduced NO production stimulated by IFN-γ at 4 weeks; the authors did not make statistical comparisons between the LPS-stimulated and IFN-γ-stimulated groups (Cohen et al., 1998).

Overall, Cr(VI) exposure had no effect on natural killer (NK) cell activity, mixed lymphocyte response (MLR), and anti-CD3 stimulation of lymphocytes in three *high* confidence drinking water studies (NTP, 2006a, b, 2005). Other studies provide some evidence for effects on mitogen-stimulated splenocyte proliferation, reactive oxygen species production, and phagocytic activity. However, data supporting effects on mitogen-stimulated splenocyte proliferation come from three *low* confidence studies (Shrivastava et al., 2005b; Snyder and Valle, 1991; Glaser et al., 1985). Data supporting effects on phagocytosis are limited to one *low* (Glaser et al., 1985) and one *medium* confidence study (Johansson et al., 1986b) whereas data on reactive oxygen species are limited to only one *low* confidence study (Cohen et al., 1998). Consequently, additional studies are necessary to better understand the potential effect of Cr(VI) on these endpoints, particularly studies that more thoroughly document exposure conditions, exposure dose, group size, data processing, and attrition.

Supportive immune system data

Immune organ pathology

No gross pathological changes were reported in six *medium* or *high* confidence NTP oral studies where rats or mice were exposed to Cr(VI) for 28 days to 2 years (NTP, 2008, 2007, 2006a, b, 2005, 1996a) and one *medium* confidence chronic inhalation study that included a 12-month recovery period (Glaser et al., 1986). In one *low* confidence drinking water study in male Wistar rats of unknown age exposed to Cr(VI) (20 mg/kg-day) for up to 135 days, evaluation of the thymus (day 90) revealed structural changes including decreased epithelial reticular cells and physiologically important associations between these cells and T cells, potentially leading to functional impairment of the central immune system (Karaulov et al., 2019). In the same study, structural effects including an increased B-zone and a decreased T-zone were observed in spleens across all timepoints (45, 90, and 135 days). Although the specific type of lymph node was not reported, lymph node size was increased and was attributed to changes in cellular elements including reticulocytes and lymphocytes.

Although unlikely to be an indicator of impaired immune function, infiltration of histiocytes (macrophages) was observed in liver, small intestine, and mesenteric and pancreatic lymph nodes in rats and mice in two *high* confidence NTP studies at oral exposure durations up to 2 years (NTP, 2008, 2007). In damaged tissues, infiltrated macrophages display functions such as modulation of inflammatory cells, removal of damaged tissues/cellular debris, and antigen presentation, as well as fibrogenic stimulation (Yamate et al., 2016). Histiocytic infiltrates were characterized by study authors as small, individual clusters and sometimes as syncytia of histiocytes that were large (approximately 20–80 microns in diameter) and had pale, lightly eosinophilic, faintly stippled

cytoplasm and single, small, peripheral, dark basophilic nuclei. This finding was distinct from the histopathological finding of chronic inflammation in the liver that NTP characterized as small, randomly scattered aggregates of macrophages, lymphocytes, and neutrophils. Dose-related findings of histiocytic infiltration were also observed in the lung following inhalation exposure (Kim et al., 2004; Glaser et al., 1990; Glaser et al., 1986; Johansson et al., 1986b; Johansson et al., 1986a) (see Section 3.2.1.2). The NTP authors (NTP, 2008, 2007) noted that the biological significance of the histiocytic cellular infiltrates is unknown but suggested this finding may indicate phagocytosis of an insoluble chemical precipitate. However, it is important to acknowledge that activated macrophages can also damage tissue by secreting cytotoxic factors indicative of an innate inflammatory response and create an inflammatory environment (Francke and Mog, 2021; Yamate et al., 2016).

Overall, Cr(VI) exposure had no effect on spleen or thymus pathology in six *medium* or *high* confidence oral studies and one *medium* quality inhalation study (28-day or 90 days with a recovery period). One *medium* quality oral study reported structural changes in the thymus and spleen and cellular content of lymph nodes after 90 days; this study did not include a recovery period, which could have increased its ability to detect effects of exposure.

Immunoglobulin levels

Short-term, subchronic and chronic inhalation exposures to Cr(VI) (25, 50 and 100 g/m^3) did not alter total serum immunoglobulin levels in one *low* confidence study performed in male Wistar rats (Glaser et al., 1990). However, in a *medium* confidence study by the same authors, Glaser et al. (1985) observed a dose-dependent increase in serum immunoglobulins in male rats following inhalation exposure for 90 days (0.025–0.10 mg/m^3); serum immunoglobulin levels returned to baseline when rats were exposed to a higher Cr(VI) concentration (i.e., 0.20 mg/m^3). Although quantitative data were not reported, serum immunoglobulins were also reported to decrease following inhalation exposure to chromium oxide for 6 months (0.1 mg/m^3) in a *low* confidence study (Glaser et al., 1986). Changes in total serum immunoglobulin levels alone are not considered sensitive enough to detect mild to moderate immunotoxicity or predictive enough to identify immunotoxicants (IPCS, 2012; Luster et al., 1993; Luster et al., 1992). However, in combination with data on measures of immune function, these results may provide supporting evidence of immunomodulation.

Immune organ weight

Absolute thymus weight was unchanged in two *high* confidence NTP studies performed in female Sprague-Dawley and F344 rats exposed to a range of Cr(VI) concentrations (5−180 mg/L) in drinking water for 28 days (NTP, 2006a, b). However, absolute thymus weight was decreased in one *high* confidence NTP study performed in male B6C3F1 and am3-C57BL/6 mice exposed to Cr(VI) (90 mg/L, high dose group only) in drinking water for 3 months (NTP, 2007). When evaluated using a higher concentration, the absolute thymus weight was unchanged in one *high* confidence NTP study performed in male and female mice and rats (B6C3F1, BALB/c, and F344/N)

exposed to a range of Cr(VI) concentrations (20–350 mg/L) in drinking water for 3 months (NTP, 2007). In one *medium* confidence study, absolute thymus weight decreased in rats exposed to chromium (20 mg/kg-d) in drinking water for up to 135 days (Karaulov et al., 2019).

NTP (2005) reported a decrease in relative spleen weight in female mice exposed to 11 mg/L Cr(VI) in drinking water for 28 days; these findings were not replicated when the study authors repeated the experiment. Relative spleen weight was not affected by exposure to Cr(VI) in drinking water for 28 days in other NTP studies (NTP, 2006a, b). However, relative spleen weight was also decreased in F344/N rats and *am3*-C57B mice subchronically exposed to chromium at doses ≥90 mg/L in drinking water (NTP, 2007). Similarly, in a *low* confidence study, relative spleen weight decreased gradually over time in mice exposed to chromium (14.8 mg/kg) in drinking water for nine weeks (Shrivastava et al., 2005b). In one *medium* confidence study, absolute spleen weight and body weight decreased in rats exposed to chromium (20 mg/kg-d) in drinking water for up to 135 days (Karaulov et al., 2019). Relative spleen weight was significantly increased in a *medium* confidence drinking water study following exposure to 50 mg/L Cr(VI) for 7 days, but not following 21 days exposure to 200 mg/L (Jin et al., 2016). These results suggest the effect may recover with time or there may be a nonmonotonic dose-response. In a *medium* confidence inhalation study, relative spleen weight increased following chromium exposure for 28 or 90 days at concentrations ≥0.050 mg/m^3 (Glaser et al., 1985). However, this effect was not observed in a *low* confidence chronic inhalation study using the same model system when the study design incorporated a 12-month recovery period following an 18-month exposure (Glaser et al., 1986). Spleen weight was also reported to be unaffected in rats exposed by inhalation to higher Cr(VI) concentrations (i.e., 0.20–1.25 mg/m^3) for 13 weeks (Kim et al., 2004).

Overall, chromium exposure only reduced absolute thymus weight in a single drinking water study and the effect was not observed in a second study exposing the same strain of mice to a broader and higher range of doses. However, absolute thymus weight was decreased in a longer duration drinking water study. Depending on the concentration of chromium tested, the exposure duration, and the route of administration, chromium exposure was shown to either have no effect, to increase, or to decrease relative spleen weight. Recognizing that immune organ weights are often confounded by stress responses, results of immune organ weight is of limited utility for immune organ pathology.

WBC counts and differentials (spleen, thymus, bone marrow)

No effects on the absolute number of splenic WBCs (total), or lymphocyte subtypes were observed in two *high* confidence NTP studies performed in female Sprague-Dawley rats and B6C3F1 mice exposed to Cr(VI) in drinking water for 28 days (5–180 mg/L) (NTP, 2006b, 2005). In another *high* confidence 28-day drinking water study in female F344 rats, the total number of splenic WBCs was also unaffected, but the numbers of NK cells and macrophages were increased at doses of 4 mg/kg-d and 0.5 mg/kg-d Cr(VI), respectively (NTP, 2006a). In both instances, the observed increase in cell number was only detected at 1 out of 4 dose levels tested in the study and

always at levels that fell within the range of concentrations tested in the other two drinking water studies (NTP, 2006b, 2005). In one *medium* confidence drinking water study in male Wistar rats exposed to Cr(VI) (20 mg/kg-day) for up to 135 days, the absolute number of splenic T cells and T helper cells was decreased on days 90 and 135, but the relative values were unaffected for these timepoints (Karaulov et al., 2019). The absolute and relative number of C8+ T cells were decreased in the spleens of rats on day 90, but not at any other timepoint. The absolute number of splenic karyocytes, and myeloid cells decreased, and effects on the absolute number of plasma cells either increased or decreased depending on the timepoint (Karaulov et al., 2019). In the same study, the absolute number of thymocytes decreased. The absolute number of bone marrow myeloid cells, neutrophils, lymphocytes, and karyocytes were increased at the 135-day timepoint (Karaulov et al., 2019).

Overall, recognizing that splenic WBC counts and differentials have only been evaluated in a small number of drinking water studies, the effects of chromium exposure on splenic WBC and splenic WBC differentials varied across studies. These differences in outcome may relate to experimental design parameters including rodent species, test article concentration and study duration. Based on a single *medium* quality study, chromium exposure has the potential to alter the number of thymocytes and bone marrow cells. Additional studies are needed to better understand the effects of chromium on WBC counts and differentials.

WBC counts (hematology)

Dose-related increases in total WBCs and some WBC types were reported in F344/N rats exposed to Cr(VI) for up to 14 weeks (NTP, 2008, 2007); however, WBC counts were similar to the control at 6 months and decreased at 12 months of exposure (NTP, 2008). Increased total WBC number was also reported in one *medium* confidence inhalation study performed in rats for 30 and 90 days but the effect reversed in animals exposed for 90 days followed by a 30-day observation period (Glaser et al., 1990). In a *low* confidence drinking water study in Swiss mice, total WBC number and some WBC types decreased after 3 weeks of Cr(VI) exposure (Shrivastava et al., 2005a).

No effects on WBCs (total or differentials) were observed in mice in three *high* confidence NTP studies (NTP, 2007, 2005, 1996a), in mice or rats in seven *medium* confidence studies (Krim et al., 2013; NTP, 2006a, b; Kim et al., 2004; Glaser et al., 1986), and in rats in two *low* confidence studies (Shrivastava et al., 2005a; Glaser et al., 1985). These short-term, subchronic, and chronic exposure studies included oral exposures via the diet (approximately 1–50 mg/kg-d Cr(VI)) (NTP, 1996a), oral gavage (5.3 mg/kg Cr(VI)) (Krim et al., 2013), and drinking water (approximately 0.5–10 mg/kg-d Cr(VI)) (NTP, 2007, 2006a, b, 2005) as well as inhalation exposures (0.025–1.25 mg/m^3) (Kim et al., 2004; Glaser et al., 1986; Glaser et al., 1985) in rats and mice. Overall, evidence for Cr(VI)-related changes in WBC count is inconsistent.

3.2.6.3. *Mechanistic and Supporting Evidence*

Available evidence from studies of apical immune endpoints in human and animals suggests that Cr(VI) exposure may have the capacity to modulate the immune system by stimulating some elements of immune responses (antibody response, mitogen-stimulated lymphocyte proliferation, total WBC counts (hematology), complement levels) and suppressing others (pathogen clearance). The sections that follow describe mechanistic data from studies of mechanistic endpoints that might inform immune effects derived from human ex vivo and in vivo animal investigations. Summary tables of mechanistic studies are presented in Appendix C.2.5.2.

Immune modulation

Several lines of mechanistic information support the conclusion that Cr(VI) exposure may have the potential to modulate the immune system. For organizational purposes, available mechanistic and supporting evidence was organized into effect categories of key characteristics common to immunotoxicants; these studies are summarized in Appendix Table C-44.

Effects on immune cell differentiation or activation

Alterations in dendric cell maturation and T cell activation could impact antigen presentation, a process central to the development of adaptive immune responses. In human monocyte-derived dendritic cells in vitro, exposure to Cr(VI) increased expression of dendritic cell maturation marker CD86 but had no effect on expression of CD83 (Toebak et al., 2006). Cr(VI) exposure decreased anti-CD3/anti-CD28-stimulated expression of T cell activation markers CD69 and CD25 in primary mouse T cells (Dai et al., 2017).

Effects on immune effector cell function

Phagocytosis is important in both innate and adaptive immune responses by removing pathogens and debris and as a key event in antigen presentation. The available animal studies (reviewed above, under "Ex vivo WBC function") reported inconsistent effects of Cr(VI) exposure on phagocytic activity (i.e., increased, decreased, or no effect) in alveolar macrophages (Johansson et al., 1986b; Glaser et al., 1985) and decreased activity in splenic macrophages (Shrivastava et al., 2005b). In vitro studies were more consistent in demonstrating that exposure to Cr(VI) decreased phagocytic activity of human PMNs isolated from workers exposed to Cr(VI) (Mignini et al., 2009), bovine alveolar macrophages (Hooftman et al., 1988), mouse peritoneal macrophages (Christensen et al., 1992), and mouse RAW264.7 macrophages (Badding et al., 2014). However, only two of these studies measured cell viability to take into account a potential role for cytotoxicity as a causative factor (Badding et al., 2014; Hooftman et al., 1988). Additional in vivo and in vitro studies would help to better understand the effects of Cr(VI) exposure on phagocytic activity.

Other in vitro studies reported diminished activity in important effector cell functions including IgG production (Borella and Bargellini, 1993), cell mobility (Christensen et al., 1992), and NK cell degranulation (Dai et al., 2017). Pokeweed mitogen-stimulated IgG production by human

primary lymphocytes was reduced by Cr(VI) exposure (Borella and Bargellini, 1993). Random cell migration was decreased in stimulated mouse primary peritoneal macrophages (Christensen et al., 1992). Activation of T cells stimulated by anti-CD3 and expression of CDa107a, a marker for NK cell degranulation, was reduced in mouse splenocytes following Cr(VI) exposure (Dai et al., 2017).

In general, although conflicting evidence was reported in the three in vivo animal studies identified, Cr(VI) exposure consistently decreased immune effector cell function in vitro. However, caution should be taken when interpreting these data, since only the studies by Badding et al. (2014), Dai et al. (2017), and Hooftman et al. (1988) evaluated cell viability as a potential causative factor for observed effects following exposure to Cr(VI).

Effects on immune cell proliferation

As discussed in the section "Ex vivo WBC function" above, the effect of Cr(VI) exposure on spleen cell proliferation ex vivo has been investigated using three approaches including mitogen stimulation, anti-CD3 ± anti-CD28 stimulation, and the MLR. Exposure to Cr(VI) in vivo increased spleen cell proliferation in the presence of ConA (Shrivastava et al., 2005b; Snyder and Valle, 1991; Glaser et al., 1985). Consistent with this finding, ConA-induced spleen cell proliferation was increased when lymphocytes collected from Cr(VI) exposed workers were cultured in the presence of Cr(IV) in vitro (Mignini et al., 2009). Furthermore, in vitro exposure to Cr(VI) increased activation by ConA in human lymphocytes, but decreased activation when exposure was to a higher dose (Mignini et al., 2009). Snyder and Valle (1991) reported inhibition of in vitro ConA-stimulated proliferation, whereas Mignini et al. (2004) reported no effect.

The effect of in vivo exposure to Cr(VI) on spleen cell proliferation stimulated by LPS has only been investigated in a single report (Snyder and Valle, 1991) (see Ex vivo WBC function). In that study, the low dose of LPS (100 mg/L), but not the high dose (200 mg/L), decreased rat splenic lymphocyte proliferation. LPS-induced spleen cell proliferation was decreased in lymphocytes cultured in the presence of Cr(IV) in vitro (Mignini et al., 2009).

Addition of Cr(VI) to lymphocytes cultured from exposed workers lead to an increase in PHA-stimulated proliferation (Mignini et al., 2009). When exposed to Cr(VI) in vitro, the proliferation response was biphasic in PHA-stimulated human primary lymphocytes (Mignini et al., 2009; Borella and Bargellini, 1993).

Cr(VI) exposure had no effect on anti-CD3 spleen cell proliferation in three rodent studies (NTP, 2006a, b, 2005). In contrast, exposure to Cr(VI) in vitro decreased anti-CD3 and anti-CD3/anti-CD28 stimulated primary human lymphocyte proliferation (Dai et al., 2017; Akbar et al., 2011).

In vivo studies showed no effect of Cr(VI) exposure on MLR (NTP, 2005; Snyder and Valle, 1991). However, MLR was increased when splenocytes collected from Cr(VI)-exposed rats were exposed to additional Cr(VI) in vitro (Snyder and Valle, 1991). When the only source of Cr(VI) exposure was in vitro, either no effect or a stimulatory effect on MLR was observed (Snyder and Valle, 1991). Recognizing that these the in vitro studies performed by were part of an investigation

(Snyder and Valle, 1991) using the same study design parameters (i.e., rat strain, exposure duration, Cr(VI) concentration, stimulator), the discrepancy may be attributable to low study replication.

Effects on communication between immune cells

Complement levels

One *low* confidence cross-sectional study investigated the effects of Cr(VI) exposure on complement levels (Table 3-32). In that study, exposure to Cr(VI) increased levels of complement C3 (mean: 0.91 ± 0.13 g/L unexposed, 1.20 ± 0.24 g/L exposed) and C4 (mean: 0.23 ± 0.05 g/L unexposed, 0.32 ± 0.07 g/L exposed) in serum (Qian et al., 2013). Serum complement levels increased two- to threefold above baseline are associated clinically with infection or acute inflammation (Ritchie et al., 2004). But even subtle increases in baseline complement C3 and C4 are associated with other inflammatory markers and have been identified as a risk factor for disorders associated with systemic inflammation, including cardiometabolic disease (Hertle et al., 2012; Engström et al., 2007b; Engström et al., 2007a; Engström et al., 2005).

Mitogen-stimulated cytokine secretion

Effects of in vivo Cr(VI) exposure on mitogen-induced cytokine secretion by isolated cells in vitro was evaluated in two *medium* confidence studies with ConA (Karaulov et al., 2019) or LPS (Cohen et al., 1998). A single in vivo study observed increased secretion of TNF-α and IL-6 in the serum of LPS challenged mice (Jin et al., 2016). There are no in vitro studies available assessing the effects of Cr(VI) exposure on ConA-stimulated cytokine secretion.

Cytokine measurements in biological media

Twenty-one studies investigated the effects of Cr(VI) on immune cell communication (see Appendix Tables C-44 and C-45). A primary mechanism of communication for cells of the immune system is through production and release of cytokines, which are low molecular weight glycoproteins involved in immune responses and are commonly classified as pro-inflammatory (i.e., immune stimulating) or anti-inflammatory (i.e., immunosuppressive). In practice, however, the distinction between the classes of cytokines is not clear cut. Interpretation of cytokine data collected from biological medium is challenging because, depending on context, the same cytokine can have either activating or suppressing effects on a particular cell type (Nature, 2019). Furthermore, reduction in the level of a pro-inflammatory cytokine can have an anti-inflammatory effect and vice versa. The effects of Cr(VI) exposure on levels of 30 cytokines (i.e., IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, IL-17A, TNF-α, IFN-γ, IFN-α, MIP-2, CXCL10, CXCL11, CCL5, CCL17, CCL18, CCL20, CCL22, eotaxin, G-CSF, GM-CSF, MCP-1, and MIP1α) have been investigated. These studies include cytokine measurements conducted following in vivo and in vitro exposures to Cr(VI) in human and animal models. Generally, the specific cytokines measured included in each study varied, making interpretation of consistency for a given cytokine difficult. Interpretation is further hampered by the mix of responses reported for the same

cytokine. Irrespective, the available data suggest that Cr(VI) exposure has the potential to alter levels of some cytokines, potentially disrupting the regulatory balance that dictates normal immune system function. While the predictive value of cytokine levels for hazard assessment is unclear, the observed alterations in cytokine levels do add to the weight of the evidence evaluation of Cr(VI) and its potential to modulate the immune system.

Vascular cell adhesion molecule 1 (VCAM-1), endothelial-leukocyte adhesion molecule 1 (ELAM-1), and intracellular adhesion molecule 1 (ICAM-1) play an important role in endothelial transmigration, the process whereby immune cells enter tissues. Expression of these important proteins is up-regulated by certain cytokines (e.g., IL-1, TNF-α). Mignini et al. (2009) reported no effect of Cr(VI) exposure had no effect on levels of these proteins.

Cr(VI) exposure had no effect on E-rosetting by human lymphocytes collected from exposed workers and treated with additional chromium in vitro. E-rosetting occurs when human T cells spontaneously bind to sheep red blood cells, a process that involves CD2 (i.e., the E-rosette receptor), which plays an important role in T cell activation.

Allergic hypersensitivity

Hypersensitivity responses are the result of an over-reaction of the immune system. Allergic hypersensitivity to Cr(VI) is generally observed following occupational exposure (Hedberg, 2018). Hypersensitivity reactions are organized into four different classes, Type I, II, III, and IV (Weaver and Murphy, 2016). There are only a few anecdotal case reports and a small number of animal studies associating Cr(VI) with Type I hypersensitivity (antibody mediated) responses that cause allergic asthma (ATSDR, 2012; Ban et al., 2010; Fernández-Nieto et al., 2006; OSHA, 2006; Bright et al., 1997; Olaguibel and Basomba, 1989); however, there is strong and compelling evidence that Cr(VI) causes Type IV hypersensitivity responses. Type IV hypersensitivity responses are mediated by T cells and are responsible for allergic contact dermatitis (ACD) resulting from dermal exposure. As described in the protocol (Appendix A), a review of the evidence for Cr(VI)-induced ACD is not included in this toxicological review because the scope of the Cr(VI) IRIS assessment is comprised of potential health effects by the inhalation and oral routes of exposure. Consequently, Cr(VI)-induced ACD was not comprehensively reviewed but was considered as supporting evidence for the effects of Cr(VI) exposure on the immune system. The strongest evidence for Cr(IV) Type IV hypersensitivity reactions comes from dermal patch testing in humans (ATSDR, 2012; OSHA, 2006). Human clinical evidence of Type IV hypersensitivity is supported by data from in vivo and ex vivo investigations performed in Guinea pigs (Wang et al., 2010a; Ikarashi et al., 1996; Helmbold et al., 1993; Saloga et al., 1988; Christensen et al., 1984; Parker et al., 1984; Jirova et al., 1983; Siegenthaler et al., 1983; Lindberg et al., 1982; Turk and Parker, 1977; Miyamoto et al., 1975; Schneeberger and Forck, 1974) and mice (Lindemann et al., 2008; Mandervelt et al., 1997; Basketter et al., 1994; Ikarashi et al., 1992; Vreeburg et al., 1991; Kimber et al., 1990; Mor et al., 1988; Lischka, 1971).

3.2.6.4. *Integration of Evidence*

Overall, the **evidence suggests** that Cr(VI) may modulate the immune system through both stimulatory and suppressive actions. This conclusion is primarily based on coherent evidence of effects on ex vivo WBC function across human and animal studies, antibody responses to T cell-dependent antigen measured in animals, and reduction in host resistance to bacterial infection reported in animal studies. However, confidence in the evidence was reduced because some of the studies are *low* confidence and reported findings often differed across studies. Integrated evidence of immune system effects of Cr(VI) exposure from human, animal, and mechanistic studies is summarized in an evidence profile table (Table 3-38).

The evidence of an association between Cr(VI) exposure and immunotoxicological effects in humans is *slight*. The available studies are *low* confidence. Data obtained from supporting immune system studies lack consistency across studies and across endpoints within studies. However, there is some evidence from the most informative studies (i.e., ex vivo WBC function) that Cr(VI) has the potential to stimulate at least some aspects of immune function. In addition, the large evidence base demonstrating that exposure to Cr(VI) can induce allergic hypersensitivity responses further supports this conclusion (ATSDR, 2012).

Evidence from animal toxicology studies and supportive mechanistic data from in vivo and in vitro studies provide *slight* evidence that Cr(VI) has both stimulatory and suppressive effects on the immune system. Cr(VI) exposure increased antibody responses to T cell-dependent antigen (i.e., sheep red blood cells), and effects on this critical function of the immune system were observed in mice exposed orally and in rats exposed orally or by inhalation (NTP, 2006a, 2005; Glaser et al., 1985). The body of evidence in support of this effect is small, but the findings are supported by evidence from some studies of increases in ex vivo WBC function (Shrivastava et al., 2005b; Cohen et al., 1998; Snyder and Valle, 1991; Glaser et al., 1985), WBC numbers (NTP, 2008, 2007; Glaser et al., 1990), and total immunoglobulin levels following in vivo Cr(VI) exposure (Glaser et al., 1985). Some mechanistic evidence has demonstrated an increased response to antigenic stimuli in one-way mixed lymphocyte cultures when splenocytes collected from Cr(VI)-exposed rats were exposed to additional Cr(VI) in vitro (Snyder and Valle, 1991) and increased mitogen-stimulated spleen cell proliferation with in vitro Cr(VI) exposure (Mignini et al., 2009; Borella and Bargellini, 1993). Data demonstrating that exposure to Cr(VI) can result in allergic hypersensitivity responses bolster these findings (ATSDR, 2012).

There is also evidence of an effect on host resistance, with short-term inhalation exposure decreasing in situ clearance of bacteria from the lungs of Cr(VI)-exposed rats (Cohen et al., 2010; Cohen et al., 2006). The host resistance model used for these studies is designed to evaluate local pathogen clearance by alveolar macrophages. While the effect cannot be directly attributed to a defect in phagocytosis, lung clearance of inhaled iron oxide was reduced in rats exposed to Cr(VI) by the inhalation route (Glaser et al., 1985). Furthermore, phagocytic activity of PMNs collected from exposed workers (Mignini et al., 2009) and splenic macrophages collected from mice exposed

to Cr(VI) in drinking water was reduced (Shrivastava et al., 2005b), and several in vitro mechanistic studies showed decreased phagocytic activity by human primary PMNs (Mignini et al., 2009), bovine alveolar macrophages (Hooftman et al., 1988), mouse peritoneal macrophages (Christensen et al., 1992), and mouse RAW264.7 macrophages (Badding et al., 2014). Cr(VI) exposure also impaired the mobility of mouse alveolar macrophages (Christensen et al., 1992). Together, these findings suggest that Cr(VI) can alter key functions of cells of the innate immune system, but additional studies would be useful for identifying the most relevant exposure contexts and the overall impact of these effects on immunity.

It is not without precedent for a single chemical to exert both stimulatory and suppressive effects on various immune parameters (IPCS, 2012). Exposure-related stimulation of the immune system might increase susceptibility to allergic disease or autoimmunity and can include exaggerated or inappropriately prolonged inflammatory responses associated with systemic chronic inflammation, which can increase risk of developing other serious health conditions such as cardiometabolic disease or cancer (Furman et al., 2019; IPCS, 2012). In addition, because continuous, uncontrolled immune stimulation represents a disruption of the homeostatic processes required to maintain a balanced immune response, stimulation of the immune system may be accompanied by immunosuppression, potentially altering host resistance as was observed here in a limited number of studies. Additional studies are necessary to better understand the effects of Cr(VI) exposure on the immune system, particularly with respect to studies of host resistance.

Table 3-38. Evidence profile table for immune effects

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
Evidence from studies of exposed humans					⊕⊙⊙ The **evidence suggests** that Cr(VI) may cause immune modulation in humans based on: *Slight* evidence from *low* confidence cross-sectional studies of workers with known risk of Cr(VI) exposure showing increased ex vivo WBC function (i.e., mitogen-stimulated proliferative responses to T cell mitogens). *Slight* evidence from *high*, *medium*, and *low* confidence studies demonstrating stimulatory effects on antibody response, ex vivo WBC function, WBC number, and Ig levels and suppressive effects on host resistance. Supportive mechanistic evidence from animal in vivo, ex vivo, and in vitro
EX VIVO WBC FUNCTION **Low confidence:** Mignini et al. (2004) Mignini et al. (2009)	Increased lymphocyte proliferation induced by two different T cell mitogens but not by a B cell mitogen. No effect on phagocytosis by PMNs or NK cell activity.	• Coherence with two different T cell mitogens • Mechanistic evidence provides biological plausibility	• *Low* confidence studies	⊕⊙⊙ *Slight* Although changes in T cell mitogen-induced lymphocyte proliferation, WBC counts, and complement factors were reported, available data were derived from *low* confidence studies.	
WBC COUNTS **Low confidence:** Boscolo et al. (1997) Sazakli et al. (2014) Wang et al. (2012a) Kuo and Wu (2002) Mignini et al. (2004) Mignini et al. (2009)	A positive association with white blood cell counts was observed in 1/3 studies, while an inverse association was also observed in 1/3 studies.	• No factors noted	• Unexplained inconsistency in WBC counts across studies • *Low* confidence studies		
WBC SUBPOPULATIONS **Low confidence:** Boscolo et al. (1997) Kuo and Wu (2002) Mignini et al. (2004) Mignini et al. (2009) Tanigawa et al. (1998)	Decreased CD4+ cell number in workers (2 of 2 studies) and in exposed and unexposed smokers and nonsmokers (1 of 1 studies). Decreased CD8+ cell number in workers (1 of 2 studies) and in exposed smokers (1 of 1 study).	• Consistent findings regarding CD4+ subpopulations in three studies	• *Low* confidence studies		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
IMMUNOGLOBULIN LEVELS **Low confidence:** Boscolo et al. (1997) Qian et al. (2013) Verschoor et al. (1988)	A consistent stimulatory effect on serum levels of IgA IgM was reported in two studies whereas effects on IgG were inconsistent in three studies.	• Consistent findings regarding serum IgA and IgM levels in two studies	• Unexplained inconsistency in IgG levels in two different studies • *Low* confidence studies		models demonstrating the potential for multiple mechanisms of immune system toxicity.
COMPLEMENT LEVELS **Low confidence:** Qian et al. (2013)	Increased complement factors C3 and C4 in one *low* confidence study.	• No factors	• *Low* confidence studies		
Evidence from animal studies					
ANTIBODY RESPONSES **High confidence:** NTP (2005) NTP (2006b) NTP (2006a) **Low confidence:** Glaser et al. (1985)	Increased IgM antibody-forming cell responses was associated with exposures in two *high* confidence drinking water studies and one *low* confidence inhalation study; the effect was not reproducible in NTP (2005), and no effect was observed in a third *high* confidence study.	• Coherence with studies performed in rats and mice following exposure via two different routes • *High* confidence studies	• Antibody response was inconsistent in *high* confidence studies	⊕⊙⊙ *Slight* Cr(VI) induced changes in the most meaningful immunological endpoints (i.e., antibody response, host resistance and ex vivo WBC function) and endpoints that provide supporting evidence (i.e., immune organ weight, immunoglobulin levels, and WBC counts).	
ANTIGEN-SPECIFIC ANTIBODY TITER **High confidence:** NTP (2005) NTP (2006b) NTP (2006a)	Three *high* confidence NTP studies of 28-day exposures in drinking water showed no effect on serum titers of total IgM antibodies specific for two different T cell-dependent antigens.	• Consistent findings regarding serum titers of IgM antibodies • *High* confidence studies	• No factors		
HOST RESISTANCE **Medium confidence:** Cohen et al. (2006)	Exposure to Cr(VI) compounds with high and low solubility was associated with decreased in situ bacterial clearance in the lung	• Consistent findings regarding in situ bacterial clearance	• No factors		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Cohen et al. (2010)		• Mechanistic evidence provides biological plausibility • *Medium* confidence studies			
EX VIVO WBC FUNCTION **High confidence:** NTP (2005) NTP (2006b) NTP (2006a) **Medium confidence:** Cohen et al. (1998) Johansson et al. (1986b) **Low confidence:** Glaser et al. (1985) Snyder and Valle (1991) Shrivastava et al. (2005b)	Effects on phagocytosis by macrophages were observed in two *low* confidence studies. Stimulatory effects on superoxide and hydrogen peroxide production were observed following 4 weeks exposure in the presence of zymosan. Cr(VI) reduced IFN-g stimulated production of nitric oxide following 4 weeks of exposure. Increased mitogen-induced proliferative response in observed in three l*ow* confidence studies. No effects on NK cell activity, the MLR, or anti-CD3-stimulated spleen cell proliferation were observed in three *high* confidence short-term drinking water studies performed in rats and mice.	• Consistent findings across studies of all confidence levels regarding NK cell activity, anti-CD3-stimulated spleen cell proliferation, MLR, and mitogen-induced proliferative response (ConA) • Coherent findings for studies investigating phagocytosis, NK cell activity, anti-CD3-stimulated spleen cell proliferation, MLR, and mitogen-induced proliferative response • Mechanistic evidence provides			

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
		biological plausibility • *High* and *medium* confidence studies			
IMMUNE ORGAN PATHOLOGY **High confidence:** NTP (1996a) NTP (2005) NTP (2007) NTP (2008) **Medium confidence:** Karaulov et al. (2019) NTP (2006b) NTP (2006a) Glaser et al. (1986)	No effects on immune organ gross pathology were reported in six medium or high confidence NTP studies and one medium confidence inhalation study. Similarly, no effects on immune organ histopathology were observed in two high confidence NTP studies. However, structural effects were reported in one medium confidence study.	• Consistent findings for immune organ gross pathology • Coherence with male and female rats and mice exposed orally or by inhalation • *High* and *medium* confidence studies	• No factors		
IMMUNOGLOBULIN LEVELS – TOTAL **Medium confidence:** Glaser et al. (1985) **Low confidence:** Glaser et al. (1986) Glaser et al. (1990)	A dose-dependent increase in serum immunoglobulins following inhalation exposure for 90 days (0.025–0.10 mg/m^3 Cr(VI)); serum immunoglobulin levels were not different from control when rats were exposed to a higher Cr(VI) concentration (i.e., 0.20 mg/m^3). Other inhalation studies of short-term, subchronic, and chronic exposure duration reported no alterations in total serum immunoglobulin levels but did not provide quantitative data.	• Dose-response gradient • *Medium* confidence study	• No factors		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
IMMUNE ORGAN WEIGHT **High confidence:** NTP (2005) NTP (2006b) NTP (2006a) NTP (2007) **Medium confidence:** Glaser et al. (1985) Jin et al. (2016) Karaulov et al. (2019) Kim et al. (2004) **Low confidence:** Glaser et al. (1986) Shrivastava et al. (2005b)	Treatment-related decrease in absolute thymus weight was only observed in some long-term studies, but not others. Effects of Cr(VI) exposure on absolute and relative spleen weight were observed in some studies, but not others. Results do not consistently correlate with dose, route of administration, exposure duration or species.	• Consistent findings regarding absolute thymus weight. • Most studies are *high* or *medium* confidence studies	• No factors		
WBC COUNTS **High confidence:** NTP (2005) NTP (2006b) NTP (2006a) **Medium confidence:** Karaulov et al. (2019)	The percent splenic macrophages increased in one *high* confidence study. The absolute number of macrophages increased, and the percentage NK cells was increased in a different *high* confidence study. The absolute and/or relative number for lymphocyte populations varied by timepoint in a *medium* confidence study. No effects on the absolute number of splenic WBCs in four drinking water studies.	• *High* and *medium* confidence studies			

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
WBC COUNTS (HEMATOLOGY) **High confidence:** NTP (1996a) NTP (2007) NTP (2008) **Medium confidence:** NTP (2005) NTP (2006b) NTP (2006a) Glaser et al. (1986) Glaser et al. (1990) Kim et al. (2004) Krim et al. (2013) Wang et al. (2015) **Low confidence:** Glaser et al. (1985) Shrivastava et al. (2005a)	Effects on WBC counts were reported in two of five studies performed in mice (4 drinking water, 1 diet) and four of nine studies performed in rats (2 drinking water, 2 inhalation). These effects were observed more often in studies of exposure durations <90 days	• Coherence, especially for rats exposed for longer exposure durations • *High* and *medium* confidence studies			
Mechanistic evidence and supplemental information					
Biological events or pathways	**Summary of key findings and interpretation**			**Judgment(s) and rationale**	
Effects on immune cell differentiation or activation	*Interpretation*: In vitro exposure studies indicate Cr(VI) has the potential to affect activation of dendritic cells, which serve an important role in innate and adaptive immune responses. Chromium exposure decreased T cell activation and proliferation in vitro. *Key findings*:			Biologically plausible, coherent and consistent observations of effects on phagocytosis.	

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
	• Dose-dependently increased expression of cell surface marker CD86 (dendritic cell maturation marker) but no effect on CD83 (activation marker for antigen presenting cells) expression in human monocyte-derived dendritic cells in vitro (Toebak et al., 2006) • Decreased activation and proliferation of T cells stimulated with anti-CD3 and anti-CD28 in vitro (Dai et al., 2017) *Limitations*: • Small evidence base • Lack of coherence between in vitro lymphocyte proliferation data (Snyder and Valle, 1991) and results of ex vivo human studies (Mignini et al., 2009; Mignini et al., 2004)			For the remaining biological events, the evidence base is too small or the findings do not align with in vivo findings (e.g., effects on cell proliferation).	
Effects on immune effector function of specific cell types	*Interpretation*: Consistent evidence that Cr(VI) decreases phagocytosis by macrophages in vitro. Phagocytosis is important in both innate and adaptive immune responses by removing pathogens and debris and also as a key event in antigen presentation. *Key findings*: • Reduced phagocytosis in PMNs collected from exposed workers and treated with additional Cr(VI) in vitro (Mignini et al., 2009) • Consistent in vitro evidence of decreased phagocytic activity by macrophages (splenic, alveolar) harvested from murine and bovine sources and by the RAW2643.7 macrophage cell line (Badding et al., 2014; Christensen et al., 1992; Hooftman et al., 1988) • Exposure to Cr(VI) in vitro had no effect on random migration in mouse primary peritoneal macrophages exposed to non-cytotoxic concentrations of Cr(VI) (Christensen et al., 1992) • In vitro evidence of decreased IgG production in human primary lymphocytes (Borella and Bargellini, 1993) • In vitro evidence of decreased cell surface expression of CD107a, a marker for NK cell degranulation (Dai et al., 2017)				

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	Limitations: • Inconsistent effects on phagocytic activity in human studies and animal studies in vivo				
Effects on immune cell proliferation	*Interpretation*: Consistent with in vivo exposure, Cr(VI) exposure in vitro has the potential to alter T cell proliferative responses. *Key findings*: • In vitro evidence of potential alterations in proliferative responses to T cell mitogens PHA and ConA (Mignini et al., 2009; Mignini et al., 2004; Borella and Bargellini, 1993; Snyder and Valle, 1991) • Cr(VI) exposure had no effect on B cell mitogen-stimulated (i.e., LPS) proliferation of lymphocytes collected from exposed workers and treated with Cr(VI) in vitro (Mignini et al., 2009) • In vitro evidence that Cr(VI) exposure decreases proliferation of lymphocytes stimulated by anti-CD3/anti-CD28 (Dai et al., 2017; Akbar et al., 2011) *Limitations*: • Inconsistent evidence for effects on the MLR • Difficulty in comparing results due to differing test conditions				
Effects on immune cell communication	*Interpretation*: Cr(VI) increases complement factors, which may indicate recent infection or development of inflammatory disease. . *Key findings:* • Cr(VI) exposure increased complement factors C3 and C4 in one *low* confidence study of serum collected from workers occupationally exposed to chromium (Qian et al., 2013) *Limitations*: • Limited evidence demonstrating effects on cell communications				

3.2.7. Male reproductive effects

The male reproductive system consists of internal and external organs that are regulated by a balanced interplay of hormones from the hypothalamus-pituitary-gonadal (HPG) axis. The development and function of the male reproductive system can be affected by toxicants that directly reach reproductive tissues or by the disruption of hormone activity at any point along the HPG axis (Creasy and Chapin, 2018). Common endpoints associated with male reproductive toxicity include semen parameters and male reproductive hormone levels in human studies, as well as changes in fertility and fecundity, sperm parameters, reproductive system organ weights and histopathology, structural abnormalities, and changes in sexual behavior in animal studies (U.S. EPA, 1996a). This section considers reproductive effects in males exposed to Cr(VI) at any life stage, including exposures occurring preconception and for all stages of development. This is in accordance with EPA's *Framework for Assessing Health Risk of Environmental Exposures To Children* (U.S. EPA, 2006d), which recommends that evidence for organ system toxicity be considered for all life stages in order to identify populations or life stages that may be more susceptible to chemical-induced toxicity. Reproductive effects resulting from developmental exposures are also considered in the "Developmental effects" section.

3.2.7.1. *Human Evidence*

Study evaluation summary

Table 3-39 summarizes the human epidemiology studies considered in the evaluation of the effects of Cr(VI) on the male reproductive system. These consist of six cross-sectional occupational studies conducted among workers in two industries with known risk of exposure to Cr(VI) in Denmark and India. They include five studies of stainless-steel welders (Danadevi et al., 2003; Hjollund et al., 1998; Bonde and Ernst, 1992; Bonde, 1990; Jelnes and Knudsen, 1988). Two of these studies were performed on the same cohort of workers using different analyses (Bonde and Ernst, 1992; Bonde, 1990) and therefore were evaluated as a single study (Table 3-39), although there are differences in the analyses and results between the two studies as discussed below. In addition, one study conducted in chromium (III) sulfate production workers was considered relevant due to evidence of exposure to Cr(VI) among the workers that could be explained by the location of the chromium sulfate operations within a chromate production plant (Kumar et al., 2005).

In all studies, the primary exposure route was inhalation of Cr(VI) in air. Air concentrations of Cr(VI) (mean [SD] = 3.6 [2.8] ug/m^3) were reported in one cohort of stainless-steel welders (Bonde, 1990) (Protocol, Section 6, Appendix A for more on consideration of welding studies in this assessment). No other studies of Cr(VI) exposure and male reproductive effects in humans reported air concentrations of Cr(VI) or total chromium.

Lack of air concentration measurements in all studies except one (Bonde, 1990) contributed to concerns about potential bias from exposure misclassification. These concerns were mitigated

when job-based dichotomous exposure categories were consistent with reported concentrations of chromium in urine (Bonde and Ernst, 1992) or blood (Danadevi et al., 2003). In one study of workers on a site where both trivalent and hexavalent chromate products were produced (Kumar et al., 2005), it is unclear whether blood concentrations of chromium reflected Cr(VI) specifically; however the high rate of nasal perforation among the workers in this study indicate a history of Cr(VI) exposure. Other study evaluation concerns included potential residual confounding (Kumar et al., 2005; Jelnes and Knudsen, 1988) and concerns about outcome measurement (Kumar et al., 2005; Hjollund et al., 1998).

The study evaluations resulted in one *medium* confidence study (Bonde and Ernst, 1992; Bonde, 1990) and four *low* confidence studies (Kumar et al., 2005; Danadevi et al., 2003; Hjollund et al., 1998; Jelnes and Knudsen, 1988). Results of the male reproductive effects in these studies—specifically, semen parameters and serum reproductive hormones—are summarized in Table 3-40.

Table 3-39. Summary of human studies for Cr(VI) male reproductive effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a] Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Study Design	Sperm Parameters	Hormones
Bonde and Ernst (1992), Bonde (1990)	SS Welding	Denmark	Cohort (occupational)	M	M
Danadevi et al. (2003)	SS Welding	India	Cohort (occupational)	L	-
Hjollund et al. (1998)	SS Welding	Denmark	Cohort (occupational)	L	U[b]
Jelnes and Knudsen (1988)	SS Welding	Denmark	Cohort (occupational)	L[c]	-
Kumar et al. (2005)	Chromium sulfate[d]	India	Cohort (occupational)	L	-

SS = Stainless Steel

[a]In addition to these included studies, two additional studies reported male reproductive outcomes that met PECO criteria but were found to be *uninformative* at the study evaluation stage: Tielemans et al. (1999); Li et al. (2001).

[b]Analysis of hormone concentrations in Hjollund et al. (1998) compared all welders to referents (no analysis comparing SS welders to referents) and therefore was found to be uninformative for this outcome.

[c]*Uninformative* for motility only. *Low* confidence for other sperm parameters.

[d]Though chromium sulfate is trivalent, there is evidence of simultaneous or recent exposure to Cr(VI) in the exposed group.

Synthesis of evidence in humans

Semen parameters

Four core endpoints were considered in the evaluation of the effects of exposure to Cr(VI) on semen parameters: volume, concentration, morphology and motility. A key consideration when assessing the quality of outcome measurements for these endpoints was the window of time following collection of samples (Radke et al., 2019). Other quality control procedures related to

collection and processing of samples were considered, including but not limited to collection of more than one sample from the same individual and abstinence period duration before sample collection.

One *medium* confidence study reported mild decreases in semen volume and sperm motility in stainless-steel welders (mean [SD] = 2.4 [1.1] mL; 51.0 [15.7] percent motile) compared to nonwelders (mean [SD] = 3.1 [1.3] mL; 57.7 [14.8] percent motile), but no differences in sperm concentration or morphology between these two groups (Bonde, 1990). In the same cohort, comparisons of sperm concentration, morphology and motility among three exposure groups characterized by urine chromium measurements were indicative of an effect but did not reach statistical significance (Bonde and Ernst, 1992) (Table 3-40). Both air concentrations and urine chromium concentration were higher among stainless-steel welders compared to mild steel welders or nonwelders, and these exposure data lent confidence to the exposure characterization of participants in both analyses. These data also reveal some exposure misclassification in both analyses that may have decreased study sensitivity. The detection of a statistically significant decrease in volume and motility despite limits to study sensitivity increased confidence in the findings of this study.

Table 3-40. Summary of results from human studies of Cr(VI) male reproductive effects

					Semen Parameters				Hormones		
Study	**Exposure**	**Confidence**	**Result Format**	**N**	**Vol (mL)**	**Concentration (million/mL)**	**% Normal forms**	**% Motile**	**T (nmol/L)**	**LH (IU/L)**	**FSH (IU/L)**
Danish Welders[a] Bonde (1990)	SS welding v. ref	M	Mean (SD) and p-value	Exp: 35 Ref: 54	Exp: 2.4 (1.1) Ref: 3.1 (1.3) $p < 0.05$	Exp: 58.4 (16.7) Ref: 58.6 (23.9) NS	Exp: 65.8 (15.7) Ref: 66.7 (17.1) NS	Exp: 51.0 (15.7) Ref: 57.7 (14.8) $p < 0.05$	Exp: 17.3 (5.8) Ref: 21.2 (8.0) $p < 0.05$	Exp: 6.1 (2.4) Ref: 7.2 (2.7) NS	Exp: 4.4 (5.1) Ref: 4.9 (2.8) NS
Danish Welders[a] Bonde and Ernst (1992)	3-level[a]	M	Unadjusted regression beta; Mean (SD) and p-value	Low: 60 Med: 24 High: 23	β: 0.2 Low: 2.9 (1.3) Med: 3.0 (1.6) High: 3.2 (1.4) NS	β: −1.5 Low: 54.5 (26.9) Med: 62.8 (21.7) High: 50.7 (20.9) NS	β: −1.6 Low: 65.8 (17.8) Med: 61.0 (17.1) High: 56.8 (20.5) NS	β: −0.5 Low: 55.2 (14.6) Med: 54.8 (11.9) High: 51.6 (16.4) NS	β: −1.2 Low: 21.0 (7.8) Med: 18.7 (7.3) High: 16.4 (5.6) NS	β: −0.1 Low: 6.8 (3.0) Med: 6.8 (2.4) High: 6.7 (2.8) NS	β: −0.1 Low: 4.7 (2.9) Med: 5.0 (2.6) High: 4.5 (2.2) NS
Danadevi et al. (2003)	Welders[b] v. Controls	L	Mean (SD) and p-value	Exp: 57 Ref: 57	Exp: 2.4 (0.5) Ref: 2.5 (0.5) NS	Exp: 14.5 (24.0) Ref: 62.8 (43.7) $p < 0.001$	Exp: 37.0 (14.3) Ref: 69.0 (8.0) $p < 0.001$	% IMMOTILE: Exp: 31.0 (16.6) Ref: 12.4 (7.0) $p < 0.001$	–	–	–
Hjollund et al. (1998)[c]	SS welding v. ref	L	Median (crude and adj)	Exp: NR Ref: NR (29, 205 respectively at enrollment)	–	Exp: 56.0 (crude) Exp: 65.5 (adj) Ref: 50.0 (crude) Ref: 46.4 (adj)	–	–	*Uninformative* for this endpoint	*Uninformative* for this endpoint	*Uninformative* for this endpoint
Jelnes and Knudsen (1988)	SS welding v. ref	L	Median and p-value	Exp: 75–77 Ref: 67–68	Exp: 3.0 Ref: 3.0 $p = 0.50–0.70$	Exp: 58.6 Ref: 58.2 $p = 0.95$	Exp: 36.0 Ref: 36.5 $p = 0.70–0.90$	*Uninformative* for this endpoint	–	–	–
Kumar et al. (2005)	Chromate workers v. ref	L	Mean (SD) and p-value	Exp: 54–61 Ref: 10–15	Exp: 2.67 (0.964) Ref: 2.54 (0.641) p = NR	Exp: 49.57 (36.3) Ref: 43.75 (29.9) p = NS	Exp: 27.87 (2.5) Ref: 45.10 (13.4) $p < 0.005$	Exp: 73.77 (11.79) Ref: 76.89 (5.76) p = NS	–	–	–

NS = not significant, as reported the study; exact p-values are included in the table when available. NR = not reported.

[a]Two analyses in the same cohort (Bonde and Ernst, 1992; Bonde, 1990). Exposure variable characterization by job category (supported by air concentration data) in 1990 analysis, exposure characterization by urine chromium (supported by job history) in 1992 analysis.

[b]Welding type not specified, blood chromium higher in welders compared to referents, co-exposure to Ni.

[c]Stainless steel and non-stainless-steel welders were pooled in the analysis of the male hormone concentrations; therefore, the hormone analysis from this study was considered *uninformative*.

Of the four other studies considered, all four measured sperm concentration and were judged to be *low* confidence for that outcome (Kumar et al., 2005; Danadevi et al., 2003; Hjollund et al., 1998; Jelnes and Knudsen, 1988) (Table 3-39). Three of the studies also measured semen volume and sperm morphology and motility and were judged to be *low* confidence for all outcomes (Kumar et al., 2005; Danadevi et al., 2003; Jelnes and Knudsen, 1988), with the exception of one study that was *uninformative* for motility (Jelnes and Knudsen, 1988). One *low* confidence study, like the *medium* confidence study discussed above, reported a statistically significant decrease in sperm concentration in occupationally exposed groups compared to referents (Danadevi et al., 2003). One study reported an increase in sperm concentration in stainless-steel workers that may have been explained by a shorter period of abstinence before sample collection in that group compared to the referent (Hjollund et al., 1998); in addition, sperm samples in this study were frozen before analysis raising concerns about the quality of the outcome measurements (WHO, 2010). In all other studies, samples were not frozen and were analyzed within a short time of collection. Also consistent with the findings of the *medium* confidence study discussed previously, two *low* confidence studies that investigated sperm motility reported decreases in the exposed group compared to referents. These findings were statistically significant in one of the studies (Danadevi et al., 2003), but did not reach significance in the other study (Kumar et al., 2005). Both studies also reported changes in morphology (i.e., decreased percent normal forms) in the occupationally exposed group compared to referents (Kumar et al., 2005; Danadevi et al., 2003). One *low* confidence study reported no effect of Cr(VI) exposure on volume, concentration, or morphology, but limited description of the methodology impeded the study evaluation (Jelnes and Knudsen, 1988).

Consistency in the findings across several of the five studies, including one *medium* confidence study, suggests that Cr(VI) exposure by the inhalation route at levels observed in occupational settings may impact semen quality. Sperm concentration, morphology, and motility were decreased in exposed groups compared to referents in three of the five studies (Kumar et al., 2005; Danadevi et al., 2003; Bonde, 1990), and these results were statistically significant for concentration (Kumar et al., 2005; Danadevi et al., 2003), morphology (Danadevi et al., 2003), and motility (Kumar et al., 2005; Danadevi et al., 2003; Bonde, 1990) despite the likely impact of exposure misclassification on study sensitivity. Evidence of a dose-response pattern to effects of Cr(VI) exposure on concentration, morphology, and motility provides further supporting evidence of a relationship between such exposures and semen quality (Bonde and Ernst, 1992). Two studies reported findings that were inconsistent with the other studies, but these may be explained by study limitations such as the use of frozen sperm samples or study quality issues (Hjollund et al., 1998; Jelnes and Knudsen, 1988). Results for semen volume were inconsistent across studies and within analyses in the same cohort, suggesting that Cr(VI) exposure is not associated with this specific endpoint.

Male hormones

The male reproductive hormones testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were considered when assessing the effects of exposure to Cr(VI) on male hormones in humans (Radke et al., 2019). The effects of Cr(VI) on other male reproductive hormones that potentially serve as endpoints for the evaluation of reproductive effects, especially for onset of puberty, such as sex hormone binding globulin and dehydroepidandrosterone (DHEA), were not investigated in the studies included in this analysis. A key consideration in the evaluation of studies of male hormones is the timing of sample collection; morning collection is recommended to account for diurnal variation in serum testosterone concentrations.

One *medium* confidence study described in two publications was considered in the evaluation of the effect of Cr(VI) exposure on male hormones (Bonde and Ernst, 1992; Bonde, 1990). A study by Hjollund et al. (1998) reported male hormones in welders and nonwelders, but the results were considered *uninformative* and are not discussed further because stainless-steel and non-stainless-steel welders were pooled in this analysis. The *medium* confidence study reported significantly decreased serum testosterone concentration in stainless-steel welders (mean [SD] = 17.3 [5.8] nmol/L) compared with nonwelders (mean [SD] = 21.2 [8.0] nmol/L) (Table 3-40) (Bonde, 1990). A dose-response dependent decrease in serum testosterone was also reported in the same cohort, though results of that analysis did not reach statistical significance (Bonde and Ernst, 1992). In the same study, decreased serum LH and FSH concentrations were also reported in stainless-steel welders compared to nonwelders, but these results did not reach statistical significance. In an alternative analysis, serum LH and FSH decreased with increased exposure to Cr(VI) characterized by urine concentration, but evidence of a dose-response trend was not as strong for these endpoints as it was for testosterone. As discussed previously in the section on semen parameters, data on air concentrations, urine chromium concentration and job history support the categorization of exposure in the *medium* confidence study; however, these data also point to exposure misclassification in both analyses that may have decreased study sensitivity. The detection of a statistically significant exposure-dependent decrease in testosterone as well as nonsignificant decreases in all three hormones measured (testosterone, LH, and FSH) despite limitations in study sensitivity increased confidence in the findings of this study.

Due to the small number of studies that assessed the relationship between Cr(VI) exposure and male reproductive hormones, consistency could not be assessed. However, evidence from two separate analyses in a *medium* confidence study indicates that exposure may impact serum concentrations of testosterone and these results are coherent with evidence for semen parameters described separately. Evidence of a relationship between Cr(VI) and serum concentration of LH and FSH was not as strong for these hormones as it was for testosterone. The *medium* confidence study found a small inverse association between Cr(VI) exposure and serum LH and FSH that was not statistically significant and was not supported by the findings of the *low* confidence study.

3.2.7.2. *Animal Evidence*

Study evaluation summary

Table 3-41 summarizes the animal toxicology studies considered in the evaluation of the effects of Cr(VI) on the male reproductive system. These consist of a two-generation reproductive study with dietary exposure using NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (NTP, 1997); subchronic oral exposure studies using diet (NTP, 1996a, b), drinking water (NTP, 2007; Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997), or gavage/unspecified oral administration (Marat et al., 2018; Rasool et al., 2014; Yousef et al., 2006); short-term exposure studies using drinking water (Wang et al., 2015) or unspecified oral administration (Kim et al., 2012); a chronic inhalation exposure study (Glaser et al., 1986); subchronic inhalation exposure studies (Kim et al., 2004; Glaser et al., 1985); and studies that evaluated F1 males that had been exposed during gestation (Zheng et al., 2018; Al-Hamood et al., 1998) or during gestation and lactation (Kumar et al., 2017). The three available inhalation studies only reported information on male gonad weights (Kim et al., 2004; Glaser et al., 1986) or histopathology (Kim et al., 2004; Glaser et al., 1985), whereas the available oral exposure studies provided more specific measurements of male reproductive function including fertility, sperm parameters, hormone levels, and sexual behavior. The report by NTP (2007) included two separate studies: a 3-month study in rats (F344/N) and mice (B6C3F1), and a second 3-month comparative study using three strains of mice (B6C3F1, BALB/c, C57BL-6).

NTP's RACB study (NTP, 1997) and subchronic exposure studies (NTP, 2007, 1996a, b) and the gestational exposure study by Zheng et al. (2018) were well-reported and well-designed to evaluate reproductive outcomes and were therefore rated as *high* confidence for almost all reported outcomes (Table 3-41). The remaining studies had reporting limitations and other substantial concerns raised during study evaluation and were rated as *low* confidence across all outcomes. Endpoint-specific concerns identified during study evaluation are discussed in the respective sections below. Three of the *low* confidence studies (Al-Hamood et al., 1998; Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997) exposed animals to high concentrations (350–1770 mg/L) of Cr(VI) in drinking water, which was considered a potential confounding variable as it is not possible to determine whether reproductive effects may have been exacerbated by reduced water consumption and/or systemic toxicity; for instance, drinking water concentrations of 350 mg/L Cr(VI) have been associated in rats with decreased water consumption and site of contact toxicity (80 and 100% incidence of ulcers in the glandular stomach of males and females, respectively) (NTP, 2007).

Table 3-41. Summary of included animal studies for Cr(VI) male reproductive effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a] Click to see interactive data graphic for rating rationales.

Author (year)	Exposure route	Species (strain)	Exposure life stage and duration	Fertility, Fecundity	Sperm evaluation	Histopathology	Hormones	Organ weights	Sexual behavior	Anogenital distance
NTP (1996a)	Diet	Mouse (BALBC)	Adult males; 3, 6, or 9 weeks	-	H	-	-	H	-	-
NTP (1996b)	Diet	Rat (Sprague-Dawley)	Adult males; 3, 6, or 9 weeks	-	H	-	-	H	-	-
NTP (1997)	Diet	Mouse (BALBC)	Reproductive Assessment by Continuous Breeding (F0 to F2)	H	H	-	-	H	-	-
NTP (2007)	Drinking water	*Study 1:* Rat (F344/N), Mouse (B6C3F1) *Study 2:* Mouse (B6C3F1, BALB/c, C57BL-6)	*Study 1:* Adult males; 3 months *Study 2:* Adult males, 3 months	-	H	M	-	H	-	-
Al-Hamood et al. (1998)	Drinking water	Mouse (BALBC)	F1 offspring; GD 12–PND 20	L	-	-	-	L	-	-
Bataineh et al. (1997)	Drinking water	Rat (Sprague-Dawley)	Adult males; 12 weeks	L	-	-	-	L	L	-
Elbetieha and Al-Hamood (1997)	Drinking water	Mouse (Swiss)	Adult males; 12 weeks	L	-	-	-	L	-	-
Kumar et al. (2017)	Drinking water	Rat (Wistar)	F1 offspring; GD 9–14	-	L	L	L	L	-	L
Wang et al. (2015)	Drinking water	Rat (Sprague-Dawley)	Adult males; 4 weeks	-	-	L	-	L	-	-
Marat et al. (2018)	Gavage	Rat (white outbred)	Adult males; 60 days	L	-	-	-	-	-	-
Yousef et al. (2006)	Gavage	Rabbit (NZ white)	Adult males; 10 weeks	-	L	-	L	L	L	-
Zheng et al. (2018)	Gavage	Rat (Sprague-Dawley)	F1 offspring; GD 12–21	-	-	H	H	-	-	-
Kim et al. (2012)	Oral (unspecified)	Rat (Sprague-Dawley)	Adult males; 6 days	-	L	-	-	L	-	-
Rasool et al. (2014)	Oral (unspecified)	Mouse (strain not reported)	Adult males; 30 or 60 days	-	-	L	-	-	-	-
Glaser et al. (1986)	Inhalation	Rat (Wistar)	Adult males; 18 months	-	-	-	-	L	-	-
Glaser et al. (1985)	Inhalation	Rat (Wistar)	Adult males; 28 or 90 days	-	-	L	-	-	-	-
Kim et al. (2004)	Inhalation	Rat (Sprague-Dawley)	Adult males; 90 days	-	-	L	-	L	-	-

GD = gestation day; PND = postnatal day.

[a]In addition to these included studies, there were seven animal toxicology studies reporting male reproductive outcomes that met PECO criteria but were found to be *uninformative* at the study evaluation stage: Aruldhas et al. (2006; 2005; 2004); Chowdhury and Mitra (1995); Li et al. (2001); Subramanian et al. (2006); Zabulyte et al. (2009); and Zahid et al. (1990).

Synthesis of evidence in animals[39,40]

Fertility and fecundity

No effects on the ability to impregnate females were observed across the five studies in rats or mice that evaluated this outcome. These consisted of the *high* confidence RACB study in mice by NTP (1997) that evaluated F0 and F1 parental animals at oral doses in diet ranging from 6.8–30.3 mg-kg/day Cr(VI) (F0) or 7.9–37.1 mg-kg/day Cr(VI) (F1); two *low* confidence studies that evaluated adult male rats or mice that had been exposed to 350 mg/L or up to 1770 mg/L Cr(VI), respectively, in drinking water for 12 weeks prior to mating (Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997); one *low* confidence study that evaluated adult male rats that had been exposed to 0.353 mg/kg-day Cr(VI) via gavage for 60 days prior to mating (Marat et al., 2018); and one *low* confidence study that evaluated adult F1 male mice that had been exposed to maternal doses of 350 mg/L Cr(VI) in drinking water during gestation and lactation (Al-Hamood et al., 1998). However, Elbetieha and Al-Hamood (1997) observed a statistically significant decrease in the number of implantations and viable fetuses when Cr(VI)-exposed male Swiss mice were mated with untreated females; this effect was observed in 710 or 1410 mg/L Cr(VI) dose groups, but not the highest dose group (1770 mg/L). Similarly, increased pre- and post-implantation mortality in rats dosed with 0.353 mg/kg-day Cr(VI) by oral gavage prior to mating was observed by Marat et al. (2018), who reported a dominant lethal mutation frequency of 0.665 by comparing the number of live fetuses in the Cr(VI) treatment group to the control group. No effects on offspring viability were observed in rats or mice in other studies following paternal exposure (Al-Hamood et al., 1998; Bataineh et al., 1997; NTP, 1997). Overall, decreased fetal viability following paternal-only exposure (indicative of dominant lethal mutations in sperm) was observed across two studies, but interpretation is limited because these studies were considered *low* confidence and the only available *high* confidence study failed to observe similar effects.

Sperm evaluation

No effects on sperm were observed in the *high* confidence subchronic exposure studies in rats and a variety of mouse strains by NTP at oral doses ranging from 0.35–32.5 mg/kg-day Cr(VI) in drinking water or diet (NTP, 2007, 1996a, b), or in the *high* confidence RACB study in mice that evaluated F0 and F1 males at doses ranging from 6.8–30.3 mg-kg/day Cr(VI) (F0) or 7.9–

[39]Data are available in HAWC for: NTP (1997) (here)
NTP (1996a) (here)
NTP (1996b) (here)
NTP (2007) (male B6C3F1 mice, male BALBC mice, male am3-C57BL/6 mice).

[40]For many of the oral studies presented here, it was not possible to estimate an average daily mg/kg dose due to lack of reporting. To estimate an average daily dose, paired records of body weight and daily intake of test article are required. This is particularly important for Cr(VI) reproductive and developmental studies, because rapid changes in maternal body weight are expected during pregnancy, and Cr(VI) affects palatability (which affects both Cr(VI) intake rate and body weight). Doses of Cr(VI) are presented where possible, however many cross-study comparisons are done on the basis of mg/L Cr(VI) in drinking water.

37.1 mg/kg-day Cr(VI) (F1) in diet (NTP, 1997). These studies reported multiple measurements aimed at evaluating effects on spermatogenesis. The NTP RACB and 3-month drinking water studies included measurements of testicular sperm head count (NTP, 2007, 1997), epididymal sperm density (NTP, 2007, 1997), epididymal sperm morphology (NTP, 1997), and evaluation of epididymal sperm motility using computer-assisted sperm motion analysis (NTP, 1997) or visual motility analysis by two observers (NTP, 2007). Sperm from both F0 and F1 males were evaluated in the RACB study (NTP, 1997). In the 3-month dietary exposure studies by NTP (1996a, b), animals underwent whole-body perfusion with fixative after 3, 6, or 9 weeks of exposure and effects on spermatogenesis were evaluated by counting the ratio of preleptotene spermatocytes and Sertoli cell nuclei in Stage X or XI tubules, with investigators blinded to the dose group. Perfusion fixation is considered the gold standard for histopathological evaluation of the testis (Haschek et al., 2009; Foley, 2001), and blinding is considered appropriate for reducing observation bias for this relatively subjective measurement. There were no notable concerns about these evaluations.

In contrast, three *low* confidence studies observed dose-related decreases in sperm quality or quantity (Kumar et al., 2017; Kim et al., 2012; Yousef et al., 2006). These studies did not indicate whether investigators were blinded during outcome evaluation and had additional reporting and study design concerns identified during study evaluation. Yousef et al. (2006) reported a statistically significant decrease in packed sperm volume, sperm concentration, total sperm output, and sperm motility, and a statistically significant increase in the percentage of dead sperm in ejaculates measured weekly from adult rabbits exposed via oral gavage to 3.6 mg/kg-day Cr(VI) for 10 weeks. Concerns were raised about the interpretation of results because the numerical data presented by the authors (means ± SE) appeared to be an average of weekly measurements across 10 weeks of exposure, which is difficult to interpret. Graphical data were shown for weekly measurements, but only as means without a measure of variance. Kumar et al. (2017) reported a statistically significant decrease in epididymal sperm forward motility (measured visually under a microscope), sperm viability, and sperm count in adult F1 rats that had been exposed during gestation at maternal doses of 17.7–70.7 mg/L Cr(VI) in drinking water. These measurements were presented as the mean of individual animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001). Kim et al. (2012) reported a statistically significant decrease in sperm head count and motility but no effect on the percentage of abnormal sperm in adult rats exposed to 10 mg/kg-day Cr(VI) for 6 days. This short exposure duration does not cover the duration of spermatogenesis, and therefore lacks sensitivity for detecting potential effects on spermatogonia. Overall, although these three studies report that Cr(VI) exposure can affect sperm quality and quantity, interpretation is limited since these studies are considered *low* confidence and higher confidence studies failed to observe any effects.

Histopathology

Almost all studies that evaluated histopathological outcomes in male reproductive tissues used conventional fixation in formalin, which is not recommended for the testis because it gives

poor penetration and may cause artifacts (Haschek et al., 2009; Foley, 2001). This was considered a sensitivity concern and reduced the confidence in this dataset. Zheng et al. (2018) is the only study that used Bouin's solution, which is considered a preferable fixative for the testis (Creasy and Chapin, 2018; Foley, 2001). The study by NTP (2007) reported that slides used for histopathological evaluation were peer reviewed and the final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Groups, which is considered a best practice for histopathological evaluations (Crissman et al., 2004). None of the other studies indicated that any steps were taken to reduce observational bias.

No dose-related lesions were observed in the testis, epididymis, prostate, or preputial gland in the 3-month drinking water exposure studies by NTP (2007) in rats and in a variety of mouse strains at oral doses up to 20.9 mg/kg-day Cr(VI) (Study 1 rats), 27.9 mg/kg-day Cr(VI) (Study 1 mice), or 8.7 mg/kg-day Cr(VI) (Study 2 mice). These studies by NTP (2007) were considered *medium* confidence for the testicular evaluation due to the use of formalin fixative and *high* confidence for other male reproductive organs. There were also no reported histopathological changes in the gonad in the *low* confidence 25- or 90-day inhalation studies in rats by Glaser et al. (1985) and Kim et al. (2004) at concentrations up to 0.2 mg/m^3 Cr(VI) or 1.25 mg/m^3 Cr(VI), respectively; or in the *low* confidence 4-week drinking water study by Wang et al. (2015) at concentrations up to 106.1 mg/L Cr(VI).

In contrast, a *high* confidence gestational exposure study (Zheng et al., 2018) and two *low* confidence subchronic oral exposure studies (Kumar et al., 2017; Rasool et al., 2014) observed histopathological changes in the testis. Zheng et al. (2018) reported altered Leydig cell distribution (increased single-cell clusters and decreased larger clusters) and decreased Leydig cell size and cytoplasmic size in F1 male rat pups following maternal exposure to 3–12 mg/kg-day Cr(VI) by oral gavage from GD 12–21, but no change in Leydig cell number or proliferation. The number of Sertoli cells and the incidence of multinuclear gonocytes in the pups was not affected. Rasool et al. (2014) observed damage to Leydig cells, germinal epithelium, and sperm cells in mice exposed to oral doses of 1.77 mg/kg-day Cr(VI) but did not provide quantitative data on the incidence and severity of the observed effects. In adult F1 male rats that had been exposed from GD 9–14 to maternal doses of 17.7–70.7 mg/L Cr(VI) in drinking water, Kumar et al. (2017) observed a statistically significant decrease in the diameter of the seminiferous tubules and lumen, number of Sertoli cells, and testicular spermatocytes and spermatids; however, this measurement was presented as the mean of individual animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001).

Within the *high* confidence study by Zheng et al. (2018), the changes in Leydig cell distribution may be coherent with the reported effects on testosterone in this study (see next section). Histopathological changes were also coherent with effects on testosterone and sperm parameters within *low* confidence studies, although the interpretation of those studies is more limited.

Hormones

Effects on reproductive hormone levels were observed across one *high* confidence and two *low* confidence studies, which were the only studies that evaluated this outcome. The *high* confidence study by Zheng et al. (2018) reported a nonmonotonic effect in which serum testosterone was increased in F1 male rat pups following maternal exposure to 3 mg/kg-day Cr(VI) by oral gavage from GD 12–21, but decreased in the 12 mg/kg-day Cr(VI) dose group. The *low* confidence study by Yousef et al. (2006) reported a statistically significant decrease in plasma testosterone in rabbits after a 12-week oral exposure to 3.6 mg/kg-day Cr(VI). Concerns about selective reporting and the presentation of results were raised because authors stated that testosterone measurements were performed biweekly but reported only a single mean value for serum testosterone. In adult F1 males that had been exposed from GD 9–14 via maternal drinking water, the *low* confidence study by Kumar et al. (2017) reported a statistically significant decrease in testosterone in serum and testicular interstitial fluid at maternal doses of 70.7 mg/L and 17.7–70.7 mg/L Cr(VI) in drinking water, respectively, and a statistically significant decrease in follicle stimulating hormone (FSH) and luteinizing hormone (LH) in serum at a maternal dose of 70.7 mg/L Cr(VI). This measurement was presented as the mean of individual animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001).

These results suggest that Cr(VI) exposure has an anti-androgenic effect at higher dose levels, although interpretation of results in the *low* confidence studies is limited. The *high* confidence studies by NTP (2007, 1997, 1996a, b) did not evaluate hormone levels, so a direct comparison with those studies is not possible; however, one mouse strain in NTP's 3-month drinking water study observed decreased testis weight (NTP, 2007), which is considered indicative of changes in androgen levels (Foster and Gray, 2013; Evans and Ganjam, 2011). The lack of effect on male reproductive organ weights in the other studies by NTP suggests that there was minimal effect on androgens on those studies.

Organ weight

Except for decreased testis weight observed in one mouse strain in the *high* confidence study by NTP (2007), effects on male reproductive organ weights were only seen in *low* confidence studies. The 3-month drinking water exposure study by NTP (2007) reported a statistically significant 11% decrease in absolute testis weight in *am3*-C57BL/6 mice in the highest dose group (8.7 mg/kg-day Cr(VI); n = 5/group). No effects were observed in the two other mouse strains (B6C3F1 and BALB/c) that were tested in this study at doses up to 8.7 mg/kg-day Cr(VI), or in F344/N rats or B6C3F1 mice at doses up to 20.9 and 27.9 mg/kg-day Cr(VI), respectively (NTP, 2007). No effects on testis or accessory reproductive organ weights were observed in the other *high* confidence RACB or 3-month dietary exposure studies in mice or rats by NTP at doses ranging from 0.35–37.1 mg/kg-day Cr(V1) (NTP, 1997, 1996a, b). There were also no effects on testis weight in

the *low* confidence studies by Glaser et al. (1986), Kim et al. (2004), Al-Hamood et al. (1998), Wang et al. (2015), or Kim et al. (2012). Kim et al. (2012) also reported no effect on epididymis weight, although the short exposure duration in this study (6 days) likely limited study sensitivity.

In contrast, four *low* confidence subchronic oral exposure studies reported Cr(VI)-induced changes in testis and accessory male reproductive organ weights. The most notable findings consisted of a statistically significant decrease in absolute testis, seminal vesicle, and preputial gland weights in rats after 12-week exposure to 350 mg/L Cr(VI) in drinking water (Bataineh et al., 1997); a statistically significant decrease in relative testis and epididymis weights in rabbits after a 10-week exposure to 3.6 mg/kg-day Cr(VI) via oral gavage (Yousef et al., 2006); and a statistically significant decrease in relative testis weight and absolute epididymal and seminal vesicle weights in adult F1 rats that had been exposed from GD 9–14 to maternal doses of 17.7–70.7 mg/L Cr(VI) in drinking water (Kumar et al., 2017). The measurements by Kumar et al. (2017) were presented as the mean of individual animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001). Additionally, the 12-week drinking water exposure study in mice by Elbetieha and Al-Hamood (1997) reported a statistically significant decrease in relative seminal vesicle and preputial gland weight in the 1770 mg/L Cr(VI) group, but a statistically significant increase in relative testis weight in the 710 and 1770 mg/L Cr(VI) groups; however, the increase in relative testis weight may have been an artifact of decreased body weight in these animals. It has been shown that testis weights are not modeled well by an organ-to-body weight ratio because testis and body weights are not proportional (Bailey et al., 2004), so relative organ weights may be a less sensitive measure than absolute testis weight.

Overall, these results suggest that male reproductive organ weights can be decreased by Cr(VI) exposure, which is consistent with decreased androgen levels as described above. However, interpretation of these results is limited because effects were predominantly observed in *low* confidence studies and were not observed in the majority of the *high* confidence studies by NTP. Effects on testis weight observed by Yousef et al. (2006) and Kumar et al. (2017) are coherent with the decreased testosterone observed in these studies.

Sexual behavior

Effects on sexual behavior were observed in two *low* confidence subchronic oral exposure studies, which were the only studies that evaluated this outcome. Neither of these studies reported that any steps were taken to reduce observational bias during outcome evaluation, which is a concern since behavior can be a relatively subjective measurement. In rats, Bataineh et al. (1997) reported a statistically significant decrease in mounts and percentage of males ejaculating, and significant increase in ejaculation latency and post-ejaculatory interval following 12 weeks of exposure to 350 mg/L Cr(VI) in drinking water. This assessment of sexual behavior was performed on a separate cohort of animals than those used in the fertility assay by these authors (see earlier section). In rabbits, Yousef et al. (2006) reported a statistically significant increase in the reaction time to mounting following 10-week exposure to 3.6 mg/kg-day Cr(VI) by oral gavage. These

results are suggestive of effects on sexual behavior, but interpretation of the results is limited because these studies are considered *low* confidence.

Anogenital distance (AGD)

The *low* confidence gestational exposure study by Kumar et al. (2017) reported a dose-related decrease in AGD in F1 male rats that had been exposed during gestation from GD 9–14 to maternal doses of 17.7–70.7 mg/L Cr(VI) in drinking water. AGD was measured at multiple timepoints between PNDs 1–30. AGD is a biomarker of androgen-dependent development, so this effect is coherent with the decreased androgen levels observed in these animals as adults (see earlier section). This measurement was presented as the mean of individual animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001). Overall, while this finding suggests that Cr(VI) exposure decreases AGD via decreased androgen levels, interpretation of the results is limited because this study is considered *low* confidence.

3.2.7.3. *Mechanistic Evidence*

The Cr(VI) literature provides evidence for potential mechanisms of Cr(VI)-induced male reproductive toxicity; specifically, oxidative stress and apoptosis in male reproductive tissues, alterations in steroid hormone signaling and the hypothalamic-pituitary-gonadal (HPG) axis, effects on the blood-testis barrier, and effects on meiosis. These studies support the biological plausibility that Cr(VI) may have the potential to act as a male reproductive toxicant acting through several possible modes of action. Mechanistic studies are tabulated in Appendix C.2.6 and summarized here.

The mechanistic studies reviewed here consisted of in vivo mechanistic data from several of the included oral exposure studies discussed above (Table 3-41), as well as from intraperitoneal (i.p.) injection studies that did not meet PECO criteria but were reviewed as informative for mechanistic analysis. Dosing via i.p. injection is likely to result in higher tissue concentrations of Cr(VI) compared to oral exposure due to the oral first-pass effect caused by the reduction of Cr(VI) in the low pH environment of the stomach; less than 10–20% of an ingested dose may be absorbed in the GI tract, and further reduction will occur in the liver prior to distribution to the rest of the body (see Section 3.1 and Appendix C). Therefore, systemic effects are expected to be more likely following i.p. injection or inhalation compared to oral exposure. Given their specific relevance to the pattern of findings observed in a subset of the in vivo animal studies, in vitro studies that evaluated Leydig, Sertoli, or male germ cells were also considered within this synthesis of mechanistic evidence.

<u>Oxidative stress</u>

Decreased antioxidant enzyme activities [e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glucose-6-phosphate

dehydrogenase (G-6-PDH), γ-glutamyl transpeptidase (γ-GT)], decreased nonenzymatic antioxidants (metallothionein, glutathione, vitamins A, C, E), and increased lipid peroxidation [measured as malondialdehyde (MDA) or lipid peroxidation potential] were observed in serum or in male reproductive tissues in rodents and monkeys concurrent with apical outcomes following oral exposure (Rasool et al., 2014; Kim et al., 2012; Subramanian et al., 2006; Aruldhas et al., 2005) or i.p. injection (El-Demerdash et al., 2019; Marouani et al., 2015a; Hfaiedh et al., 2014; Acharya et al., 2006; Acharya et al., 2004). Similar markers of oxidative stress were observed in vitro in cultured mouse Leydig cells, Sertoli cells, or spermatagonial stem cells (Lv et al., 2018; Das et al., 2015). Although antioxidant levels were generally decreased across studies, increased GST or metallothionein were observed in some cases (Das et al., 2015; Marouani et al., 2015a; Aruldhas et al., 2005), indicating an antioxidant response.

Several in vivo studies demonstrated that effects on sperm, testicular histopathology, hormones, and male fecundity were attenuated following cotreatment with antioxidants (El-Demerdash et al., 2019; Lv et al., 2018; Hfaiedh et al., 2014; Kim et al., 2012; Subramanian et al., 2006). This may imply that oxidative stress is a mechanism underlying these effects, but interpretation is difficult because antioxidants can also decrease tissue Cr(VI) levels by stabilizing lower Cr oxidation states. For instance, Subramanian et al. (2006) reported lower plasma Cr levels with coadministration of Vitamin C. The authors hypothesized that the protective effect of Vitamin C may be due to enhanced conversion of Cr(VI) to Cr(III).

Apoptosis of somatic and germ cells

Increased expression of the pro-apoptotic protein BAX and increased DNA fragmentation (measured using DNA ladders or by the biomarker γ-H2AX) were observed in the testes of male rats and mice following i.p. injection (Lv et al., 2018; Marouani et al., 2015a). I.p. injection studies have also reported degenerative histopathological changes in seminiferous tubules and spermatogenic cells, absence of spermatocytes in the seminiferous tubules, and lower sperm counts in rats, mice, and rabbits (El-Demerdash et al., 2019; Lv et al., 2018; Acharya et al., 2004; Behari et al., 1978).

In vitro studies using mouse Leydig, Sertoli, or spermatagonial stem cells provided additional evidence of the activation of intrinsic (mitochondria-dependent) apoptotic pathways, including increased staining in the TUNEL assay, decreased mitochondrial membrane potential, decreased BAX/BCL-2 ratio, and increased cleavage of caspases 3 and 9 in all three of these cell types (Lv et al., 2018; Das et al., 2015). In vitro studies also found that biomarkers of extrinsic apoptosis (caspase 8, Fas) were not activated, further supporting intrinsic apoptosis as the mechanism of cell death (Lv et al., 2018; Das et al., 2015). It was demonstrated both in vivo and in vitro that apoptosis was attenuated following cotreatment with an antioxidant (Lv et al., 2018; Das et al., 2015).

Altered steroidogenesis and effects on the HPG axis

As described above, a *high* confidence oral gavage study in rats reported a nonmonotonic effect on fetal testosterone in F1 male rats (increased at the lowest dose and decreased at the highest dose) (Zheng et al., 2018), and two *low* confidence oral exposure studies in rabbits (Yousef et al., 2006) and rats (Kumar et al., 2017) reported decreased testosterone and gonadotropin levels. Zheng et al. (2018) also reported nonmonotonic or decreased mRNA and/or protein expression of genes involved in testicular steroidogenesis and differentiation. The mRNA and protein expression changes were generally consistent with the observed effects on testosterone, although some steroidogenic genes [e.g., CYP11A and steroidogenic acute regulatory protein (StAR)] were not affected. Similarly, i.p. injection studies reported decreased testosterone (El-Demerdash et al., 2019; Hfaiedh et al., 2014; Marouani et al., 2012), decreased LH, and increased FSH (El-Demerdash et al., 2019; Marouani et al., 2012) in adult male rats. Hfaiedh et al. (2014) and El-Demerdash et al. (2019) found that the hormone changes were attenuated by cotreatment with an antioxidant. An in vitro study in cultured mouse Leydig cells reported decreased testosterone secretion and decreased transcriptional expression of genes in the steroidogenesis pathway after Cr(VI) treatment (Das et al., 2015). In cultured mouse Sertoli cells, the same study reported decreased transcriptional expression of androgen receptor (Ar) and follicle stimulating hormone receptor (Fshr), both of which play a key role in the maturation and functioning of Sertoli cells (Das et al., 2015). The transcriptional changes in the in vitro studies are coherent with the anti-androgenic effects observed in the available in vivo studies.

Another series of studies specifically suggested that the pituitary and hypothalamus were targeted by Cr(VI). Male rats exposed to 73.05 mg/kg-day Cr(VI) for 30 days by drinking water were found to have Cr accumulation in the pituitary and decreased serum prolactin, but no effect on serum LH, with the same trend observed in primary rat anterior pituitary cells treated with Cr(VI) in vitro (Quinteros et al., 2007). A follow-up study using the same experimental design but lower dose [11.6 mg/kg-day Cr(VI)] reported accumulation of Cr and evidence of oxidative stress in the pituitary and hypothalamus (Nudler et al., 2009). Oxidative stress and apoptosis were also reported in primary anterior pituitary cells treated with Cr(VI) in vitro and were mitigated by cotreatment with an antioxidant (Quinteros et al., 2008; Quinteros et al., 2007).

Effects on blood-testis barrier

Several studies reported that Cr(VI) exposure impaired the dynamics of the blood-testis barrier. In rats exposed by i.p. injection, Murthy et al. (1991) observed leakage of Sertoli cell tight junctions and adverse effects on late stage spermatids using electron microscopy. In cultured mouse Sertoli cells in vitro, Cr(VI) treatment decreased transcriptional expression of tight junction signaling molecules (Das et al., 2015). Comparatively, in a bicameral chamber culture of rat primary Sertoli and germ cells that maintains the blood-testis barrier, gap junction coupling was decreased

and the gap junction protein connexin 43 was delocalized from the membrane to the cytoplasm, but adherins and tight junction proteins were not affected (Carette et al., 2013).

Effects on meiosis

A single study provides evidence of an effect of Cr(VI) on meiosis, another potential mechanism for effects on spermatogenesis. Using the same bicameral culture chamber model as Carette et al. (2013), Geoffroy-Siraudin et al. (2010) observed that Cr(VI) treatment decreased the number of late spermatocytes and round spermatids and increased the percentage of cells with alterations in meiotic prophase.

3.2.7.4. *Integration of Evidence*

Overall, the **evidence suggests** that Cr(VI) may cause male reproductive toxicity under relevant exposure circumstances. This conclusion is based on coherent evidence of effects across human and animal studies. Decreased testosterone and decreased sperm quantity and quality were observed in both human and animal studies; however, interpretation of this evidence was limited because most studies that observed these effects were considered *low* confidence and there was inconsistency with higher confidence studies. Integrated evidence of the male reproductive effects of Cr(VI) exposure from human, animal, and mechanistic studies is summarized in an evidence profile table (Table 3-42).

The evidence of an association between Cr(VI) exposure and male reproductive effects in humans is *slight* and indicated by an inverse association between occupational exposure to Cr(VI) and several sperm parameters (concentration, morphology and motility) as well as an inverse association between exposure and serum testosterone concentrations. This is largely based on a single *medium* confidence study in welders (Bonde and Ernst, 1992; Bonde, 1990) and supported by some coherent findings from *low* confidence studies. Evidence of a dose-response pattern in these associations further supports this conclusion. Though some results did not reach statistical significance, this may be explained by the likely impact of exposure misclassification on study sensitivity in all available studies.

Evidence from animal toxicology studies and supportive mechanistic data from in vivo and in vitro studies provide *slight* evidence that Cr(VI) is a male reproductive toxicant. Findings from *high* confidence drinking water and dietary exposure studies by NTP that exposed rats or mice as adults (NTP, 2007, 1996a, b) or for multiple generations using an RACB design (NTP, 1997) indicate that the male reproductive system is not responsive to Cr(VI)-induced toxicity following oral exposure, with no observed effects on sperm parameters, histopathological outcomes, or male fertility or fecundity. In contrast, a *high* confidence gestational exposure study in which maternal rats were dosed by oral gavage[41] reported alterations in testosterone and Leydig cell size and

[41]As previously noted, oral gavage administration is likely to achieve higher systemic absorption of un-reduced Cr(VI) than ad libitum drinking water or dietary administration.

distribution, and the available *low* confidence developmental and subchronic oral exposure studies reported effects including decreased male fecundity (suggestive of dominant lethal mutations in sperm), decreased sperm quantity and quality, decreased testosterone and gonadotropins, decreased male reproductive organ weights, and altered mating behavior. These *low* confidence studies had multiple deficiencies regarding study design, conduct, and reporting. Support for biological plausibility of Cr(VI)-induced male reproductive toxicity is provided by mechanistic data demonstrating evidence of oxidative stress and apoptosis in male reproductive tissues and pituitary, altered steroid hormone signaling, disruption of the blood-testis barrier, and alterations in meiosis, although much of this evidence was derived from i.p. injection studies and in vitro studies that have unclear relevance for other routes of exposure.

In the only human study that provided a quantitative measure of Cr(VI) exposure (Bonde, 1990), effects were observed at air mean (SD) concentrations of 3.6 (2.8) $\mu g/m^3$; these reported concentrations may underestimate exposure in this study population due use of a cellulose fiber filter during sampling, which can contribute to reduction of Cr(VI) to Cr(III). In animal toxicology studies, the observation of decreased testis weight occurred at 8.7 mg/kg-day Cr(VI) in the 3-month drinking water study in mice by NTP (2007), and effects were observed at doses of 3–12 mg/kg-day Cr(VI) (Zheng et al., 2018), 0.353 mg/kg-day Cr(VI) (Marat et al., 2018), or 3.6 mg/kg-day Cr(VI) (Yousef et al., 2006) in oral gavage studies. For the other drinking water studies in animals, the doses of Cr(VI) at which effects were observed could not be calculated because drinking water consumption data was not reported. Effects were not observed in any of the three animal studies that evaluated inhalation exposure, but those studies did not include specific measures of male reproductive structure and function, so were considered insensitive. There is therefore inadequate information to evaluate the extent of effects in oral versus inhalation exposure.

Table 3-42. Evidence profile table for male reproductive outcomes

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from studies of exposed humans					⊕⊙⊙ The **evidence suggests** that Cr(VI) causes male reproductive toxicity in humans. Effects on sperm parameters and testosterone were observed in both human and animal studies. Most human and animal studies were considered *low* confidence. Effects in *low* confidence animal studies were generally not seen in the *high* confidence RACB and subchronic studies by NTP. Mechanistic findings (animals and in vitro) provide evidence supportive of male reproductive toxicity. These mechanisms are presumed relevant to humans.
SPERM PARAMETERS ***Medium* confidence:** Bonde et al. (1992; 1990) Low confidence: Danadevi et al. (2003) Hjollund et al. (1998) Jelnes and Knudsen (1988) Kumar et al. (2005)	Note: Sperm concentration was measured in all five studies considered; other endpoints were measured in some but not all of the studies. Decreased sperm motility in 1 medium study and 2 low confidence studies (1 statistically significant at $p < 0.001$, 1 no p-value or significance reported); a fourth study was uninformative for this measurement. Decreased % sperm with normal morphology in 2 low confidence studies (out of 4 studies), and decreased sperm concentration in 1 low confidence study (out of 5 studies). Decreased semen volume was reported in 1 medium confidence study, but no effect on volume was reported in 3 low confidence studies.	• Consistency • Dose-response gradient • Detection of effects despite limitations to study sensitivity • Mechanistic evidence provides biological plausibility	• High proportion of *low* confidence studies • Bias from exposure misclassification and reduced study sensitivity may have impacted ability to detect an effect	⊕⊙⊙ *Slight* Occupational (inhalation) Cr(VI) exposure is inversely associated with sperm concentration, normal sperm morphology, sperm motility, and serum testosterone. These findings are consistent and coherent across multiple studies and endpoints, but interpretation is limited because most studies evaluating sperm were considered *low* confidence. Evidence of the impact of Cr(VI) exposure on semen volume and serum LH and FSH concentrations in humans is unclear.	
HORMONES ***Medium* confidence:**	Exposure associated with decreased serum testosterone concentration in Danish stainless-	• Dose-response gradient	• Uncertainty about exposure measurements		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Bonde et al. (1992; 1990)	steel welders. Decreases in serum LH or FSH concentrations that were not statistically significant were also reported.	• Mechanistic evidence provides biological plausibility	due to multiple factors that impact exposure among welders.		
Evidence from animal studies					
FERTILITY AND FECUNDITY ***High* confidence:** NTP (1997) ***Low* confidence:** Al-Hamood et al. (1998) Bataineh et al. (1997) Elbetieha and Al-Hamood (1997) Marat et al. (2018)	No effects on ability to impregnate females. Decreased fetal viability (indicative of dominant lethal effects) in two low confidence studies in rats and mice following paternal-only exposure; no effects on fetal viability in other three studies.	• No factors noted	• Effects observed only in *low* confidence studies	⊕⊙⊙ *Slight* Evidence of male reproductive effects was observed primarily in *low* confidence studies (drinking water or gavage) and in one *high* confidence gavage study. *High* confidence RACB and subchronic studies by NTP observed no male reproductive effects, aside from decreased testis weight in one mouse strain. Evidence was insufficient to evaluate the extent of effects following inhalation exposure.	
SPERM EVALUATION ***High* confidence:** NTP (1996a) NTP (1996b) NTP (1997) NTP (2007) ***Low* confidence:** Kim et al. (2012) Kumar et al. (2017) Yousef et al. (2006)	No effects on sperm parameters in four high confidence studies in rats or mice, including an RACB study (F0 and F1 males) and three 3-month exposure studies. Low confidence studies in rabbits and F1 rats report decreased sperm quality and quantity.	• No factors noted	• Effects observed only in *low* confidence studies		
HISTOPATHOLOGY ***High* confidence:** NTP (2007) Zheng et al. (2018)	No dose-related lesions in male reproductive tissues in a high confidence 3-month drinking	• *High* confidence study • Dose-response gradient	• No factors noted		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
***Low* confidence:** Kumar et al. (2017) Rasool et al. (2014)	water study in rats and a variety of mouse strains. A high confidence gestational exposure study in F1 rats reported Leydig cell alterations. Two low confidence studies in rats and mice observed histopathological changes in the testis and seminiferous tubules.	• Coherent with effects on testosterone			
HORMONES ***High* confidence:** Zheng et al. (2018) Low confidence: Kumar et al. (2017) Yousef et al. (2006)	Nonmonotonic effect on serum testosterone in a high confidence gestational exposure study in F1 rats. Decreased testosterone in two low confidence studies in adult rabbits and F1 rats.	• *High* confidence study • Coherent with effects on Leydig cells • Mechanistic evidence provides biological plausibility	• No factors noted		
ORGAN WEIGHT ***High* confidence:** NTP (1996a) NTP (1996b) NTP (1997) NTP (2007) ***Low* confidence:** Al-Hamood et al. (1998) Bataineh et al. (1997) Elbetieha and Al-Hamood (1997) Glaser et al. (1986)	Decreased testis weight in one mouse strain in the high confidence 3-month drinking water study by NTP (2007). Changes (typically, decrease) in testis and accessory male reproductive organ weights in 4 low confidence studies in rabbits, rats, and mice. No effects observed in other mouse strains evaluated in NTP	• *High* confidence study • Coherent with decreased testosterone within *low* confidence studies	• Unexplained inconsistency across *high* confidence studies		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Kim et al. (2004) Kim et al. (2012) Kumar et al. (2017) Wang et al. (2015) Yousef et al. (2006)	(2007), or in any of the remaining studies.				
SEXUAL BEHAVIOR ***Low* confidence:** Bataineh et al. (1997) Yousef et al. (2006)	Decreased mounts, increased ejaculation latency and post-ejaculation interval, and decreased percentage of males ejaculating in rats exposed as adults. Increased reaction time to mounting in rabbits.	• No factors noted	• *Low* confidence studies		
ANOGENITAL DISTANCE **Low confidence:** Kumar et al. (2017)	Decreased AGD in developing F1 males.	• No factors noted	• *Low* confidence study		
Mechanistic evidence					
Biological events or pathways	**Summary of key findings and interpretations**			**Judgments and rationale**	
Oxidative stress	*Interpretation:* In vivo and in vitro evidence of Cr(VI)-induced oxidative stress in male reproductive tissues or in serum concurrent with effects on sperm or testicular pathology. *Key findings:* • Across most studies, decreased antioxidant activity or expression in male reproductive tissues or serum observed in animals exposed orally (Rasool et al., 2014; Kim et al., 2012; Subramanian et al., 2006; Aruldhas et al., 2005) or i.p. (El-Demerdash et al., 2019; Marouani et al., 2015a; Hfaiedh et al., 2014; Acharya et al., 2006) and in cultured mouse Leydig, Sertoli, and spermatogonial stem cells (Lv et al., 2018; Das et al., 2015)			Observations of oxidative stress, apoptosis, altered steroid hormone signaling/effects on the HPG axis, effects on the blood-testis barrier, and alterations in meiosis. Oxidative stress was concurrent with apical	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	• Consistent observation of increased testicular or epididymal lipid peroxidation in animals exposed orally (Rasool et al., 2014; Kim et al., 2012) or i.p. (El-Demerdash et al., 2019; Marouani et al., 2015a; Hfaiedh et al., 2014; Acharya et al., 2006; Acharya et al., 2004), and increased reactive oxygen species in vitro (Lv et al., 2018; Das et al., 2015) • Cotreatment of with antioxidants mitigated effects on sperm, testicular histopathology, male hormones, and male fecundity in Cr(VI)-exposed animals (El-Demerdash et al., 2019; Lv et al., 2018; Hfaiedh et al., 2014; Kim et al., 2012; Subramanian et al., 2006), and decreased Cr(VI)-induced apoptosis in vitro (Lv et al., 2018; Das et al., 2015)			outcomes in some animal studies. Testicular degeneration, decreased testosterone, and apoptosis are mitigated by cotreatment with antioxidants. Much of this evidence was derived from i.p. injection studies and in vitro studies that have unclear relevance for other routes of exposure.	
Apoptosis of somatic and germ cells	*Interpretation:* In vivo and in vitro evidence of Cr(VI)-induced apoptosis in male reproductive tissues. *Key findings:* • In vivo expression of BAX and DNA fragmentation in testes following i.p. injection (Lv et al., 2018; Marouani et al., 2015a) • Degenerative changes in testis and decreased sperm counts in animals after i.p. injection (El-Demerdash et al., 2019; Lv et al., 2018; Acharya et al., 2004; Behari et al., 1978) • In vitro evidence of intrinsic apoptosis (TUNEL staining, decreased mitochondrial membrane potential, decreased BAX/BCL-2 ratio, and increased cleavage of caspases 3 and 9) in cultured Leydig, Sertoli, and spermatogonial stem cells (Lv et al., 2018; Das et al., 2015)				
Altered steroid hormone signaling and effects on the HPG axis	*Interpretation:* Cr(VI) alters steroidogenesis in vivo and in vitro. *Key findings:* • Decreased testosterone and altered gonadotropin levels in animals following oral (subchronic and gestational) (Kumar et al., 2017; Yousef et al., 2006) and i.p. exposures (El-Demerdash et al., 2019; Hfaiedh et al., 2014; Marouani et al., 2012)				

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	• Biphasic effects on testosterone in one oral exposure study (increased at lowest dose and decreased at highest dose), supported by changes in expression of steroidogenic genes and proteins in testis (Zheng et al., 2018) • Oxidative stress in pituitary and hypothalamus and decreased prolactin secretion in rats following 30-day oral exposure (Nudler et al., 2009; Quinteros et al., 2007) and in cultured rat primary anterior pituitary cells (Quinteros et al., 2008; Quinteros et al., 2007) • Decreased testosterone production and transcriptional expression of steroidogenic genes in cultured Leydig and Sertoli cells in vitro (Das et al., 2015)				
Effects on blood-testis barrier	*Interpretation:* In vivo and in vitro evidence of impaired dynamics of the blood-testis barrier. *Key findings:* • Leakage of Sertoli cell tight junctions and adverse effects on late stage spermatids in rats exposed i.p. (Murthy et al., 1991) • Changes in the expression of molecules that form the blood-testis barrier in rat Sertoli cells (Das et al., 2015; Carette et al., 2013)				
Effects on meiosis	*Interpretation:* In vitro evidence of impaired meiosis. *Key findings:* • Evidence of impaired meiotic prophase in a bicameral culture chamber model using rat primary Sertoli and germ cells (Geoffroy-Siraudin et al., 2010)				

3.2.8. Female reproductive effects

Female reproductive effects include endpoints related to the structure and function of reproductive organs in pregnant and non-pregnant females, and the balance and cycling of hormones from the HPG axis that regulate the development and function of these organs. This section considers reproductive effects in females exposed to Cr(VI) at any life stage, including exposures occurring preconception and for all stages of development. This is in accordance with EPA's *Framework For Assessing Health Risk of Environmental Exposures To Children* (U.S. EPA, 2006d), which recommends that evidence for organ system toxicity be considered for all life stages in order to identify populations or life stages that may be more susceptible to chemical-induced toxicity. Exposure during pregnancy can affect both the mother and the fetus, and it is frequently not possible to determine whether effects on the fetus are in response to or separate from maternal toxicity in studies that report both. The maternal endpoints in animal toxicology studies described in this section (maternal body weight gain and gestation length) must therefore be considered in conjunction with the fetal endpoints (survival, growth, and structural alterations) that are discussed in the Developmental Effects Section, 3.2.9.

3.2.8.1. *Human Evidence*

Exposure to Cr(VI) in the general population is not as well characterized as occupational exposure, where men predominate, and thus limited data is available on female reproductive effects. One human epidemiology study (Remy et al., 2017) considered female reproductive effects of Cr(VI) exposure. The single available study was an ecologic study of a population living near a factory that used Cr(VI) in their production processes and where there was documented contaminated groundwater. This study was considered *low* confidence due to potential for exposure misclassification from the ecologic design (exposure was based on location of residence in relation to the factory), outcome misclassification, and confounding. This study reported higher relative risk of reproductive organ neoplasm (RR 1.27, 95% CI: 1.08, 1.5), pelvic inflammatory disease (1.31 (1.17,1.47)), endometriosis (1.19 (1.05, 1.36)), menstrual disorder (1.15 (1.03, 1.29)), and ovarian cyst (1.43 (1.23, 1.65)) in the more exposed geographic area. Overall, due to concerns for potential bias, these data are difficult to interpret on their own.

3.2.8.2. *Animal Evidence*

Study evaluation summary

Table 3-43 summarizes the animal toxicology studies considered in the evaluation of the effects of Cr(VI) on the female reproductive system. These consist of a two-generation reproductive study with dietary exposure using NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (NTP, 1997); subchronic oral exposure studies in adult animals (Thompson et al., 2020; NTP, 2007; Kanojia et al., 1998; Elbetieha and Al-Hamood, 1997; Murthy et al., 1996; NTP, 1996a,

b); gestational exposure studies that were designed to evaluate offspring development but also reported some F0 maternal outcomes, such as gestational weight gain (Zheng et al., 2018; Samuel et al., 2012a; Elsaieed and Nada, 2002; Junaid et al., 1996b, 1995; Trivedi et al., 1989); and studies that evaluated effects in F1 females from dams that had been exposed during gestation or lactation (Banu et al., 2016; Banu et al., 2015; Sivakumar et al., 2014; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a; Banu et al., 2008; Al-Hamood et al., 1998).

The RACB study (NTP, 1997) and subchronic exposure studies by NTP (2007, 1996a, b) were well-reported and well-designed to evaluate reproductive outcomes and were therefore rated as *high* confidence for all reported outcomes (Table 3-43). The subchronic exposure study in mice by Thompson et al. (2020) was also rated as *high* or *medium* confidence for most outcomes. The remaining studies had reporting limitations and other substantial concerns raised during study evaluation and were rated as *low* confidence across almost all outcomes. Endpoint-specific concerns are discussed in the respective sections below. Two of the *low* confidence studies (Al-Hamood et al., 1998; Elbetieha and Al-Hamood, 1997) exposed animals to high concentrations (350–1770 mg/L) of Cr(VI) in drinking water, which was considered a potential confounding variable as it is not possible to determine whether reproductive effects may have been exacerbated by reduced water consumption and/or systemic toxicity; for instance, drinking water concentrations of 350 mg/L Cr(VI) have been associated in rats with decreased water consumption and site of contact toxicity (80 and 100% incidence of ulcers in the glandular stomach of males and females, respectively) (NTP, 2007). There were concerns about scientific integrity for two groups of authors[42] (Banu et al., 2016; Banu et al., 2015; Sivakumar et al., 2014; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a; Banu et al., 2008; Kanojia et al., 1998; Junaid et al., 1996b; Murthy et al., 1996; Junaid et al., 1995; Trivedi et al., 1989), which reduces confidence in these studies but does not necessarily discount the results.

[42]Four studies demonstrating self-plagiarism—i.e., publication of identical data presented as separate and unique experiments—were considered *uninformative* and were excluded from the assessment. Specifically, 1) identical data were presented for rats by Kanojia et al. (1996) and for mice by Junaid et al. (1996a), despite these being presented as separate studies in different species; and 2) subsets of the data presented by Samuel et al. (2012b; 2011) were identical to that in an earlier publication by this laboratory group (Banu et al., 2008). Other studies by the same groups of authors, listed in the text above, were included in the assessment but considered *low* confidence.

Table 3-43. Summary of included studies for Cr(VI) female reproductive effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a] Click to see interactive graphic with ratings rationale.

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Fertility, Fecundity	Maternal BW gain	Gestation length	Hormones	Estrous cyclicity	Timing of puberty	Organ weight	Oocytes/ovarian histopathology	Other
NTP (1996a)	Mice (BALBC)	Adult females; 3, 6, or 9 weeks	Diet	-	-	-	-	-	-	-	H	H
NTP (1996b)	Rat (Sprague-Dawley)	Adult females; 3, 6, or 9 weeks	Diet	-	-	-	-	-	-	-	H	H
NTP (1997)	Mice (BALBC)	Reproductive Assessment by Continuous Breeding (F0 to F2)	Diet	H	H	H	-	H	-	H	H	H
NTP (2007)	Rats (F344/N); Mice (B6C3F1)	Adult females; 3 months	Drinking water	-	-	-	-	-	-	-	H	H
Al-Hamood et al. (1998)	Mice (BALBC)	F1 females; GD 12–PND 20	Drinking water	L	-	-	-	-	L	L	-	-
Banu et al. (2008)	Rat (Wistar)	F1 females; PND 1–21	Drinking water	-	-	-	L	L	L	-	L	-
Banu et al. (2015)	Rat (Sprague-Dawley)	F1 females; GD 9.5–14.5	Drinking water	-	-	-	-	-	-	-	L	-
Banu et al. (2016)	Rat (Sprague-Dawley)	F1 females; PND 1–21	Drinking water	-	-	-	L	-	-	-	L	-
Elbetieha and Al-Hamood (1997)	Mice (Swiss)	F0 dams; 12 weeks prior to mating	Drinking water	L	-	-	-	-	-	L	-	-
Elsaieed and Nada (2002)	Rat (Wistar)	F0 dams; GD 6–15	Drinking water	-	L	-	-	-	-	-	-	-
Junaid et al. (1995)	Mice (Swiss albino)	F0 dams; GD 14–19	Drinking water	-	L	-	-	-	-	-	-	-
Junaid et al. (1996b)	Mice (Swiss albino)	F0 dams; GD 6–14	Drinking water	-	L	-	-	-	-	-	-	-
Kanojia et al. (1998)	Rat (Druckrey)	F0 dams; 3 months prior to mating	Drinking water	L	L	-	-	L	-	-	L	-
Murthy et al. (1996)	Mice (Swiss)	Adult females; 20 or 90 days	Drinking water	-	-	-	-	L	-	-	L	-
Samuel et al. (2012a)	Rat (Wistar)	*Study 1*: F0 dams and F1 females; GD 9–21 *Study 2*: F1 females; GD 9–PND 65	Drinking water	-	-	-	L	L	L	L	L	-
Sivakumar et al. (2014)	Rat (strain not reported)	F1 females; GD 9.5–14.5	Drinking water	L	-	-	-	-	-	-	L	-
Stanley et al. (2013)	Rat (Sprague-Dawley)	F1 females; PND 1–21	Drinking water	-	-	-	L	-	-	-	L	-
Stanley et al. (2014)	Rat (Sprague-Dawley)	F1 females; PND 1–21	Drinking water	-	-	-	L	-	L	-	L	-
Thompson et al. (2020)	Mice (B6C3F1)	5-week-old females; 90 days	Drinking water	-	-	-	-	L	-	H	H	M

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Fertility, Fecundity	Maternal BW gain	Gestation length	Hormones	Estrous cyclicity	Timing of puberty	Organ weight	Oocytes/ovarian histopathology	Other
Trivedi et al. (1989)	Mice (albino)	F0 dams; GD 0–19	Drinking water	-	L	-	-	-	-	-	-	-
Zheng et al. (2018)	Rat (Sprague-Dawley)	F0 dams; GD 12–21	Gavage	-	L	-	-	-	-	-	-	-

BW = body weight; GD = gestation day; PND = postnatal day

[a]In addition to these included studies, there were four animal toxicology studies reporting female reproductive effects that met PECO criteria but were found to be *uninformative* at the study evaluation stage: Junaid et al. (1996a), Kanojia et al. (1996), Samuel et al. (2011), and Samuel et al. (2012b).

Synthesis of evidence in animals[43] [44]

Fertility and fecundity

In the *high* confidence RACB study in mice (NTP, 1997), Cr(VI) exposure did not affect pregnancy index in F0 females at doses up to 50 mg/kg-day Cr(VI) via diet, and had no effect on mating index, pregnancy index, or fertility index in F1 females at doses up to 39 mg/kg-day Cr(VI) via diet. Additionally, no effects on pregnancy rate were observed in the *low* confidence study by Elbetieha and Al-Hamood (1997), in which mice were exposed to 707–1770 mg/L Cr(VI) in drinking water for 12 weeks prior to mating with untreated males.

In contrast, the *low* confidence study by Kanojia et al. (1998) reported a decrease in mating index and fertility index in female rats exposed to 88.4–265 mg/L Cr(VI) in drinking water for 3 months prior to mating with untreated males. Two *low* confidence gestational exposure studies also observed decreased pregnancy rates in F1 females from dams exposed to 8.8 mg/L Cr(VI) in drinking water from GD 9.5–14.5 (rats) (Sivakumar et al., 2014) or 353 mg/L Cr(VI) in drinking water from GD 12–PND 20 (mice) (Al-Hamood et al., 1998). Both of the gestational exposure studies evaluated the F1 animals as individuals without considering the effects of litter, which has

[43]For many of the oral studies presented here, it was not possible to estimate an average daily mg/kg dose due to lack of reporting. To estimate an average daily dose, paired records of body weight and daily intake of test article are required. This is particularly important for Cr(VI) reproductive and developmental studies, because rapid changes in maternal body weight are expected during pregnancy, and Cr(VI) affects palatability (which affects both Cr(VI) intake rate and body weight). Doses of Cr(VI) are presented where possible, however many cross-study comparisons are done on the basis of mg/L Cr(VI) in drinking water. Reporting and nomenclature related to exposure concentration units and water intakes for the studies by Kanojia et al. (1998), Murthy et al. (1996), and Junaid et al. (1995) were inconsistent with each other. This assessment assumes that the drinking water concentrations provided by these studies (from the same laboratory) were in units of mg/L potassium dichromate.

[44]Data are available in HAWC for:
NTP (1997) (here)
NTP (1996a) (here)
NTP (1996b) (here).

the potential to overestimate statistical significance (Haseman et al., 2001). Additionally, there is uncertainty about how pregnancy rates were determined in the study by Sivakumar et al. (2014), which bred the animals continuously for 8–10 months and presented data as the percentage of F1 females pregnant at various blocks of age (2–4, 4–6, 6–8, and 8–10 months old); the authors did not indicate how many times the animals became pregnant within each of these 2-month windows or provide any additional information on how these percentage were calculated. Overall, although decreased fertility was observed across several studies, interpretation is limited because these studies were considered *low* confidence.

Maternal body weight gain

Decreased maternal body weights at the time of delivery were observed for both F0 and F1 dams in the RACB study in mice (NTP, 1997), which was considered *high* confidence for this outcome. For F0 dams, which were allowed to produce up to five litters, the trend was statistically significant for the first four litters; dam body weights were statistically significantly 5% decreased compared to controls at doses of 24.4 mg/kg-day Cr(VI) for the first litter and 5–7% decreased compared to controls at 50.6 mg/kg-day Cr(VI) for the first, second, and third litters, but were not statistically significantly different from the control group in the fourth or fifth litters. For F1 dams, the trend towards decreased dam body weights was statistically significant but treated animals did not differ significantly from controls in any dose group. This study also observed a trend towards decreased F0 dam body weights during lactation for the final litter; this trend was statistically significant at PNDs 1, 4, and 14, and dam body weights were statistically significantly different from controls at doses of 24.4–50.6 mg/kg-day Cr(VI) at these timepoints.

Dose-dependent decreases in maternal gestational weight gain were also observed in five *low* confidence studies in which F0 rats or mice were exposed to potassium dichromate in drinking water and sacrificed near the end of gestation. None of these studies adjusted for gravid uterine weight, which is considered preferable in order to distinguish between maternal and fetal toxicity (U.S. EPA, 1991), so the magnitude of decreased gestational weight gain in these *low* confidence studies likely reflects a combination of maternal toxicity as well as the decreased fetal growth and survival that was observed in these studies (see "Developmental effects" section). Kanojia et al. (1998) exposed female rats for 90 days prior to mating and reported that gestational weight gain was decreased by 10–22% compared to controls in the 88–265 mg/L dose groups, reaching statistical significance at 177 mg/L Cr(VI). A 10–15% mortality rate and clinical signs of hair loss and lethargy were also noted in females in the 177 and 265 mg/L dose groups in this study. In three studies by the same group of authors that exposed mice for various durations during pregnancy, gestational weight gain was decreased compared to controls by 11–26% (Junaid et al., 1995), 8–24% (Junaid et al., 1996b), and 17–20% (Trivedi et al., 1989) following exposure from GDs 14–19, 6–14, and 0–19, respectively, reaching statistical significance at 177 mg/L Cr(VI) in all studies with no mortality or clinical signs of toxicity observed. The study by Trivedi et al. (1989) included a high dose group of 354 mg/L Cr(VI) in which the dams lost weight during the treatment period and did

not produce any litters. Elsaieed and Nada (2002) exposed rat dams to 50 mg/L Cr(VI) from GD 6–15 and observed a 40% decrease in maternal body weight gain.

Lastly, in the *low* confidence study by Zheng et al. (2018), no effect on maternal body weight was observed in F0 rat dams exposed from GD 12–21 at oral gavage doses up to 12 mg/kg-day Cr(VI); however, body weight measurements in this study were taken 10 days after the exposure ended, so are potentially insensitive due to the lag time between the exposure and endpoint evaluation.

Gestation length

The only study that evaluated effects on gestation length was the *high* confidence RACB study in mice by NTP (1997). There was no effect on the cumulative days to litter for F0 dams over the course of five litters at doses up to 50.6 mg/kg-day Cr(VI) via diet. "Cumulative days to litter" is the number of days from cohabitation to the birth of each litter and is used as a metric for gestation length in the RACB in lieu of checking for a copulatory plug. For F1 dams in this study, which were only allowed to produce one litter and were checked for copulatory plugs to confirm mating, there was likewise no effect on gestation length at doses up to 39 mg/kg-day Cr(VI) via diet.

Hormones

Statistically significant decreases in serum estrogen, testosterone, and progesterone were observed in weanling and peripubertal F1 females in four *low* confidence studies in which F0 dams were exposed to 17.7–70.7 mg/L Cr(VI) in drinking water during lactation (PND 1–21) (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Banu et al., 2008). The same effects as well as decreases in prolactin and growth hormone were observed in F1 females in the *low* confidence study by Samuel et al. (2012a), in which F0 dams were exposed to 70.7 mg/L Cr(VI) in drinking water from GD 9–PND 21 and F1 females were continued on the same dosing regimen from weaning through PND 65. Three of these studies also evaluated gonadotropins and observed a statistically significant increase in follicle stimulating hormone (Stanley et al., 2013; Samuel et al., 2012a; Banu et al., 2008). Luteinizing hormone was statistically significantly increased in the study by Samuel et al. (2012a), whereas it was not affected in the study by Banu et al. (2008). Across all five studies, effects were observed at all tested doses and generally at all timepoints evaluated, which ranged from PND 0–65. Although results were consistent across studies, it should be noted that all five studies were performed by the same group of researchers, so it is unclear whether results would be replicated by an outside research group or by higher confidence studies. Measurements in all studies were presented as the mean of individual animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001). Samuel et al. (2012a) reported that body weights were decreased in the F1 females, whereas the other studies did not report whether there was an effect on body weight or other evidence of overt toxicity coinciding with the hormonal effects. Overall, the results indicate that

Cr(VI) decreases sex steroid hormone levels in females exposed during development, but interpretation is limited because all studies were considered *low* confidence.

Estrous cyclicity

There were no notable effects on estrous cycle length, number of cycles, relative time spent in estrous stages, or number of females with regular cycles in F1 mice in the *high* confidence dietary exposure RACB study by NTP (1997). The proportion of F1 females with irregular cycles increased with dose from 0/20 in the control group to 3/20 in the 39 mg/kg-day Cr(VI) dose group, but this effect was not statistically significant and the remaining females had regular cycles with lengths between 4–5 days. There was also no apparent effect on estrous cyclicity in mice exposed to levels up to 149.3 mg/L Cr(VI) in drinking water for 90-days in a study by Thompson et al. (2020); however, the authors did not provide quantitative data and based their conclusion on a single vaginal smear taken at study termination, so the study was considered *low* confidence for this outcome.

Four *low* confidence studies reported statistically significant increases in estrous cycle length. A direct comparison between results from these *low* confidence studies and NTP (1997) is complicated by the difference in oral administration (feed vs. drinking water), and inadequate reporting of body weights and/or drinking water consumption by the *low* confidence studies (precluding estimates of the mg/kg-d doses[45]). In adult rats exposed for 90 days, estrous cycle duration was dose-dependently increased from a mean of 5.15 days in control animals to 8.66 days at 265 mg/L Cr(VI) (Kanojia et al., 1998); however, effects above 88.4 mg/L Cr(VI) may be related to overt toxicity, as there was a 10–15% mortality rate and decreased body weight among females in the 177 and 265 mg/L dose groups. In another study in adult mice that used these same dose levels but a 20-day exposure duration, there was a statistically significant increase in estrous cycle duration from a mean of 4.4 days in control animals to 7.7 days at 265 mg/L Cr(VI) with no effects at lower dose levels (Murthy et al., 1996). The authors did not report whether there was an effect on body weights or clinical signs of toxicity, which are likely to occur at the 265-mg/L dose level and limits the interpretation of this finding. The remaining two studies investigated estrous cyclicity in F1 females that had been exposed during development. Samuel et al. (2012a) exposed F0 dams to 70.7 mg/L Cr(VI) in drinking water during gestation and lactation (GD 9–PND 21) and continued F1 females on the same dosing regimen through PND 65 and observed a statistically significant increase in the number of hours spent in metestrous and diestrous by the F1 animals. Similarly, Banu et al. (2008) reported a statistically significant increase in the number of hours spent in diestrous for F1 females from dams exposed to 70.7 mg/L Cr(VI) in drinking water from PND 1−21, but no change in other estrous phases. None of the available studies indicated whether

[45]Based on the information available, the *ad libitum* drinking water doses from Kanojia et al. (1998) and Murthy et al. (1996) were higher than the dietary doses from NTP (1997), while the doses in Banu et al. (2008) and Samuel et al. (2012a) were lower than NTP (1997).

investigators were blinded to treatment groups during the evaluation of vaginal cytology, which would be considered appropriate for reducing observational bias. Measurements in the developmental exposure studies by Samuel et al. (2012a) and Banu et al. (2008) were presented as the mean of individual F1 animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001). The finding of increased estrous cycle duration is coherent with the decreased expression of sex steroid hormones within the developmental studies by Samuel et al. (2012a) and Banu et al. (2008) (see "Hormones" section above), but interpretation is limited because effects were observed only in low confidence studies.

Timing of puberty

Four *low* confidence studies that evaluated F1 females following developmental exposure reported a statistically significant increase in the age at vaginal opening, which is a biomarker of female puberty. In F1 mice from dams exposed to potassium dichromate in drinking water from GD 12–PND 20, Al-Hamood et al. (1998) observed a statistically significant increase in the mean age of vaginal opening from 24.6 days in control animals to 27 days at 353 mg/L Cr(VI); however, the authors did not report whether there was overt maternal toxicity, which would be expected at this high dose level (see "Maternal body weight gain" section above) and could limit the interpretation of this finding. In two studies that exposed rat dams to potassium dichromate in drinking water from PND 1–21, there were statistically significant increases in the mean age of vaginal opening in F1 females from 33 days in control animals to 55 days at 70.7 mg/L Cr(VI) (Banu et al., 2008), and from 31 days in control animals to 42 days at 17.7 mg/L (Stanley et al., 2014). Another study in developing rats by Samuel et al. (2012a) exposed F0 dams to 70.7 mg/L Cr(VI) in drinking water during gestation and lactation (GD 9–PND 21) and continued F1 females on the same dosing regimen through PND 65, and observed a statistically significant increase in the mean age of vaginal opening from 42.3 days in control animals to 65 days at 70.7 mg/L Cr(V)[46]. In all four of these studies, results were presented as the mean of individual F1 animals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001).

Delayed puberty is coherent with decreased estrogen levels in three of these studies (Stanley et al., 2014; Stanley et al., 2013; Banu et al., 2008) (see "Hormones" section above). Delayed puberty can also be closely tied to decreased body weight (Greenspan and Lee, 2018), so examination of body weight may provide a means for separating direct effects on puberty from those that are related to general delays in development. Samuel et al. (2012a) reported decreased body weights in Cr(VI) treatment groups at multiple postnatal timepoints, whereas Banu et al. (2008) and Stanley et al. (2014) did not report body weights. Al-Hamood et al. (1998) reported that body weight of the F1 females was not affected by Cr(VI) exposure, but the study was not clear

[46]Numerical values in the study by Samuel et al. (2012a) were extracted from a figure using WebPlotDigitizer software: https://automeris.io/WebPlotDigitizer/.

about when the body weight measurements were taken. Thus, the delayed puberty could be related either to decreases in reproductive hormones or body weight. Overall, interpretation of these *low* confidence studies is limited.

Organ weight

Effects on female reproductive organ weight were inconsistent across studies. No effects on absolute or relative ovary weights were observed in adult F0 or F1 females in the *high* confidence RACB study in mice at doses up to 50.6 and 39 mg/kg-day Cr(VI) via diet, respectively (NTP, 1997). The *high* confidence study by Thompson et al. (2020) reported no change in the absolute weight of the ovaries or uterus following a 90-day exposure to 149.3 mg/L Cr(VI) in drinking water. No effect on relative ovary or uterus weights were observed at PND 50 in F1 female mice exposed to 353 mg/L Cr(VI) in drinking water from GD 12–PND 20 in the *low* confidence developmental exposure study by (Al-Hamood et al., 1998). In the *low* confidence study in adult mice by (Elbetieha and Al-Hamood, 1997), relative ovary weight was statistically significantly increased following exposure to 1770 mg/L Cr(VI) in drinking water for 12 weeks, while relative uterus weight was not changed. Conversely, in the *low* confidence study in rats by Samuel et al. (2012a), there was a dose-dependent decrease in absolute uterus and ovary weight in F0 rat dams exposed to potassium dichromate in drinking water from GD 9–21 that reached statistical significance at 35.3 mg/L and 70.7 mg/L Cr(VI), respectively. The study by Samuel et al. (2012a) also evaluated F1 females that were continued on the 70.7 mg/L Cr(VI) dosing regimen through PND 65, and observed a statistically significant decrease in absolute ovary and uterus weight at multiple timepoints measured between PND 3 and PND 65. Samuel et al. (2012a) evaluated F1 animals as individuals without accounting for potential litter effects, which has the potential to overestimate statistical significance (Haseman et al., 2001). Body weights were decreased in both studies that observed effects, which could have contributed to the increase in relative organ weights and decrease in absolute organ weights. Overall, interpretation is limited because effects were only observed in *low* confidence studies and were not seen in *high* confidence studies, and the direction of effect was inconsistent.

Oocytes and ovarian histopathology

The *high* confidence subchronic studies by NTP reported no gross or microscopic changes in the ovary in adult rats or mice following up to 9 weeks of exposure to doses up to 8.5 or 32.5 mg/kg-day Cr(VI) via diet, respectively (NTP, 1996a, b); or in adult rats or mice following 3-month exposure to doses up to 20.9 or 27.9 mg/kg-day Cr(VI) via drinking water (NTP, 2007), respectively. No gross changes were observed in the ovary in F0 or F1 females in the *high* confidence RACB study in mice at doses up to 50.6 and 39 mg/kg-day Cr(VI) via diet, respectively (NTP, 1997). The *high* confidence study by Thompson et al. (2020) likewise reported no change in the numbers of small, medium, or large follicles and no change in the incidence of follicular atresia in mice following 90-day exposure to levels up to 149.3 mg/L in drinking water.

In contrast, nine *low* confidence studies reported pathological effects in the ovary following exposure to potassium dichromate in drinking water. Kanojia et al. (1998) reported a statistically significant decrease in the number of corpora lutea in maternal female rats that had been exposed to doses of 177 mg/L Cr(VI) and higher in drinking water for 3 months prior to mating; however, there was a 10–15% mortality rate and clinical signs of toxicity among rats at these dose levels, so this effect may be indicative of overt toxicity. Similarly, following exposure in adult mice for 20 days, Murthy et al. (1996) reported a dose-related statistically significant decrease in follicle numbers at drinking water concentrations of 88.4 mg/L Cr(VI) and higher, and a statistically significant decrease in the number of ova recovered when the animals were induced to superovulate at concentrations of 177 mg/L Cr(VI) and higher. The remaining seven *low* confidence studies evaluated ovarian histopathology in developing F1 females and were performed by a single group of authors (Banu, Stanley, Sivakumar, Samuel, and coauthors). Following gestational exposure (GD 9.5–14.5) of F0 dams to 8.8 mg/L Cr(VI), F1 female rat fetuses and newborn pups were found to have decreased oocyte counts and accelerated breakdown of germ cell nests into primordial follicles[47] (Banu et al., 2015; Sivakumar et al., 2014), with an increased number of primary and secondary follicles at PND 4 in treated animals compared to the control group (Banu et al., 2015). Following lactational exposure (PND 1–21) of F0 dams to 8.8–70.7 mg/L Cr(VI), F1 female rats were found to have a dose-related increase in incidence of follicular atresia[48] (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013) and decreased numbers of primordial, primary, secondary, and antral follicles (Banu et al., 2008) at timepoints between PND 21 and PND 65. Samuel et al. (2012a) exposed F0 dams to 70.7 mg/L Cr(VI) in drinking water during gestation and lactation (GD 9–PND 21) and continued F1 females on the same dosing regimen through PND 65, and observed pyknotic nuclei and vacuolation in oocytes, stunted or arrested ovarian follicle development, and abnormalities in thecal cells, granulosa cells, and luteum in F1 females at various timepoints measured between PND 3–65, but did not provide quantitative data. These ovarian effects are coherent with the effects on hormones that were observed in some of these studies (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a; Banu et al., 2008) (see above section) since estrogens and gonadotropins play a critical role in the growth and development of oocytes. However, interpretation is limited because effects were observed only in *low* confidence studies and were not seen in the *high* confidence studies.

Other histopathology of the female reproductive system

The *high* confidence studies by NTP reported no effects on the incidence of gross or microscopic lesions in the vagina, cervix, uterus, or clitoral gland in adult rats or mice following up to 9 weeks of exposure to doses up to 8.5 or 32.5 mg/kg-day Cr(VI) via diet, respectively (NTP,

[47]Germ cell nests are clusters of oogonia that are formed in the developing ovary during late gestation. Germ cell nests are present at birth, and then are broken down into primordial follicles during the final stage of early ovarian development (Wear et al., 2016).
[48]Follicular atresia is defined as degenerative changes in the granulosa cell layers or oocyte.

1996a, b); or in adult rats or mice following 3-month exposure to doses up to 20.9 or 27.9 mg/kg-day Cr(VI) via drinking water (NTP, 2007), respectively. No treatment-related gross lesions were observed in these organs in F0 or F1 females in the RACB study in mice at doses up to 50.6 and 39 mg/kg-day Cr(VI) via diet, respectively (NTP, 1997). The study by Thompson et al. (2020) likewise reported no significant alterations in the gross and microscopic appearance of the corpus and cervix uteri, vaginas, or mammary glands, but was considered *medium* confidence for this outcome because no quantitative data was reported.

3.2.8.3. *Mechanistic Evidence*

The Cr(VI) literature provides evidence informing potential mechanisms of Cr(VI)-induced female reproductive toxicity; specifically, oxidative stress and apoptosis in female reproductive tissues, altered hormone signaling, and effects on the extracellular matrix. Mechanistic studies are tabulated in Appendix C.2.7 and summarized here.

The mechanistic studies reviewed here consisted of in vivo mechanistic data from several of the included oral exposure studies discussed above (Table 3-43), as well as from intraperitoneal (i.p.) injection studies that did not meet PECO criteria but were reviewed as relevant to the mechanistic synthesis. Dosing via i.p. injection is likely to result in higher tissue concentrations of Cr(VI) compared to oral exposure, since an oral first-pass effect exists due to the reduction of Cr(VI) in the low pH environment of the stomach; less than 10–20% of an ingested dose may be absorbed in the GI tract, and further reduction will occur in the liver prior to distribution to the rest of the body (see Section 3.1 and Appendix C.1). Therefore, systemic effects are expected to be more likely following i.p. injection or inhalation compared to oral exposure. In vitro studies conducted in relevant cell types, such as thecal and granulosa cells, were also considered for mechanistic evidence.

Altered steroidogenesis

The effects on hormone levels (described in sections above) are supported by changes in the ovarian expression of genes involved in steriodogenesis, which were observed in rats and rat granulosa cells following exposure to potassium dichromate. In F1 rats, Stanley et al. (2013) reported decreased ovarian FSH receptor gene expression and Banu et al. (2016) reported decreased ovarian gene expression of steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase, and aromatase. Banu et al. (2016) also reported increased gene expression of enzymes involved in the metabolic clearance of estradiol (Cyp1a1, Cyp1b1, UDP-glucuronosyltransferases, Sult1a1, NAD(P)H quinone oxidoreductase 1). Similar effects were observed in an immortalized rat granulosa cell line (Stanley et al., 2011; Banu et al., 2008) and in primary rat granulosa cells (Stanley et al., 2013; Stanley et al., 2011), including decreased expression of LH receptor, FSH receptor, estrogen receptors (ERα, ERβ), StAR, steroidogenic factor (SF)-1, and 17β-hydroxysteroid dehydrogenases -1 and -2. In all of these studies, these effects (including steroid hormone measurements in the in vivo studies) were attenuated by cotreatment

with an antioxidant (vitamin C or resveratrol). Stanley et al. (2014) found that cotreatment of potassium dichromate-exposed F1 female rats with estradiol restored the expression of several antioxidant enzymes (Gpx1, catalase, Prdx3, and Txn2), also suggesting a relationship between hormonal effects and oxidative stress.

Oxidative stress

Decreased antioxidant enzyme expression or activity [e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), peroxiredoxin (PRDX) 3, and thioredoxin (TXN)], decreased nonenzymatic antioxidants (glutathione, metallothionine, vitamin C), and increased markers of oxidative stress (lipid peroxidation, superoxide anion, H_2O_2) were observed in the ovary in several of the studies in F1 rats described above (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a) and in adult mice (Rao et al., 2009) following oral exposure, as well as in the uterus of adult rats following intraperitoneal injection (Marouani et al., 2015b). Increased ovarian glutathione-S-transferase (GST) (Stanley et al., 2013) and SOD expression (Banu et al., 2016) were observed in some cases. A similar spectrum of effects was observed in vitro in primary granulosa and theca cells isolated from immature rats and in an immortalized granulosa cell line (Stanley et al., 2013). Sivakumar et al. (2014) observed that potassium dichromate exposure increased colocalization of p53/SOD-2 in the ovary of F1 rats and hypothesized that this could be contributing to oxidative stress, as p53 has been demonstrated to reduce SOD-2 antioxidant activity.

Several in vivo studies found that cotreatment of animals with antioxidants (vitamin C, resveratrol, ginseng edaravone) mitigated apical outcomes including decreased maternal body weight gain, follicular atresia, and effects on pubertal onset, estrous cyclicity, and hormone levels (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Banu et al., 2008; Elsaieed and Nada, 2002). This may imply that oxidative stress is a mechanism underlying these effects, but interpretation is difficult because antioxidants can also decrease tissue Cr(VI) levels by stabilizing lower Cr oxidation states. For instance, (Elsaieed and Nada, 2002) reported lower plasma, placenta, and fetus Cr levels with coadministration of ginseng, and (Banu et al., 2008) reported lower plasma and ovarian Cr levels with coadministration of Vitamin C.

Apoptosis of somatic and germ cells

In rat studies that reported follicular atresia and decreased follicle counts in F1 pups (Banu et al., 2016; Banu et al., 2015; Sivakumar et al., 2014; Stanley et al., 2014; Stanley et al., 2013), these histopathological changes were accompanied by increased apoptosis of follicular cells. Evidence included increased staining in the TUNEL assay, increased expression of pro-apoptotic markers (Bax, cytochrome c, caspase-3, p53, p27), decreased expression of anti-apoptotic markers (Bcl-2, Bcl-XL, Bcl2l1, HIF-1α), and decreased expression of other signaling molecules that regulate cell survival [p-AKT, p-ERK, X-linked inhibitor of apoptosis protein (XIAP)]. Increased apoptotic cells and protein expression of Bax in the uterus was also reported in adult female rats following

intraperitoneal injection with potassium dichromate, accompanied by a decrease in the relative weight of the uterus and ovary (Marouani et al., 2015b). In primary granulosa cells from immature rats, (Banu et al., 2011) similarly reported upregulation of apoptotic markers and down-regulation of anti-apoptotic markers and further investigated the role of signal transduction pathways that regulate cell survival, finding that apoptosis and p53 activity were decreased after treatment with an ERK1/2 inhibitor. Another study in primary and immortalized rat granulosa cells reported that potassium dichromate induced cell cycle arrest, decreased expression of proteins that regulate the progression of the cell cycle [cyclins, cyclin-dependent kinases (CDKs), and proliferating cell nuclear antigen (PCNA)], and increased expression of inhibitors of CDKs (p15, p16, and p27), although authors stated it was unclear whether these disruptions to the cell cycle were a cause or a consequence of apoptosis (Stanley et al., 2011).

Effects on the ovarian extracellular matrix

Banu et al. (2015) proposed a mechanism by which Cr(VI) induces premature ovarian failure by targeting the metalloenzyme X-propyl aminopeptidase (coded by the gene Xpnpep2), leading to effects on the extracellular matrix. In F1 female rats from dams that were exposed to 25 mg/L potassium dichromate in drinking water from GD 9.5–14.5, the authors reported increased ovarian expression of Xpnpep2 during late gestation and decreased ovarian expression of Xpnpep2 during early postnatal life. Levels of ovarian collagen expression (Col1, Col3, Col4) were inversely proportional to Xpnpep2 at each of the sample time points. The authors hypothesized that Cr(VI) accelerates the breakdown of germ cell nests by upregulating Xpnpep2 and decreasing the distribution of collagen in the fetal ovary and alters the histoarchitecture of the ovary in postnatal animals by downregulating Xpnpep2.

3.2.8.4. *Integration of Evidence*

Overall, the available **evidence is inadequate** to assess whether Cr(VI) may cause female reproductive effects under relevant exposure circumstances. Although an association with female reproductive toxicity was demonstrated in a single *low* confidence epidemiology study and a series of *low* confidence animal toxicology studies, effects were not observed in *medium* or *high* confidence studies aside from a moderate decrease in maternal body weight (NTP, 1997). Integrated evidence of the female reproductive effects of Cr(VI) exposure from human, animal, and mechanistic studies is summarized in an evidence profile table (Table 3-44).

The evidence of an association between Cr(VI) exposure and female reproductive effects in humans is *indeterminate*. A single *low* confidence study indicated higher risk of several female reproductive conditions in a population that was estimated to have higher Cr(VI) exposure, but there is too much uncertainty to draw conclusions regarding these associations.

Evidence of female reproductive effects from animal toxicology studies and supportive mechanistic data from in vivo and in vitro studies was also found to be *indeterminate*. Across *high* confidence studies in rats and mice (Thompson et al., 2020; NTP, 1997, 1996a, b), the only notable

female reproductive effect was a 5–7% decrease in F0 and F1 maternal body weights at delivery in the RACB study in mice (NTP, 1997); fertility, fecundity, and estrous cyclicity were not affected, and effects on organ weights, follicle counts, and histopathology were not observed. In contrast, profound effects on female fertility, estrous cyclicity, hormone levels, ovarian follicles, and reproductive development were observed across the other available studies, which were all considered *low* confidence and many of which were from a single research group. The doses of Cr(VI) at which effects were observed could not be calculated for any of the *low* confidence studies because drinking water consumption data was not reported, but the available information indicates that some were higher and some were lower than doses used by NTP; so, it is unlikely that the discrepancy in responses between *high* and *low* confidence studies is simply due to a difference in the dose ranges tested. Some of the *low* confidence studies used relatively high dose levels associated with mortality or other overt toxicity, however, which limits the interpretation of the female reproductive findings. A strength of these *low* confidence studies is that they evaluated several indicators of female reproductive toxicity that were not included in the NTP studies: specifically, steroid hormone and gonadotropin levels, age at pubertal development, and ovarian histopathology during early developmental stages. The interpretation of the *low* confidence studies is limited, however, by deficiencies in study design, conduct, and reporting. Support for biological plausibility of Cr(VI)-induced female reproductive effects comes from mechanistic data that was also largely published by the same laboratory group, demonstrating altered expression of steroid hormone signaling pathways in female rats and rat cells, as well as oxidative stress and apoptosis in rodent ovarian and uterine tissues and cells. There were no animal studies that evaluated female reproductive effects following inhalation exposure.

Table 3-44. Evidence profile table for female reproductive outcomes

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from studies of exposed humans					⊙⊙⊙ The **evidence is inadequate** to assess whether Cr(VI) causes female reproductive toxicity in humans. The single human study and most animal studies were considered *low* confidence. With the exception of decreased maternal body weight, effects in *low* confidence animal studies were not seen in the *high* confidence RACB and subchronic and studies. Mechanistic findings (animals and in vitro) provide evidence supportive of female reproductive toxicity. These mechanisms are presumed relevant to humans.
FEMALE REPRODUCTIVE EFFECTS **Low confidence:** Remy et al. (2017)	One ecologic study reported higher relative risk for reproductive organ neoplasm, pelvic inflammatory disease, endometriosis, menstrual disorder, and ovarian cysts in a higher exposed geographic area.	• No factors noted	• *Low* confidence study	⊙⊙⊙ *Indeterminate* There is some indication of an association between Cr(VI) exposure and female reproductive effects, but the only evidence comes from a single, *low* confidence ecologic study so there is considerable uncertainty in the findings.	
Evidence from animal studies					
FERTILITY AND FECUNDITY **High confidence:** NTP (1997) **Low confidence:** Kanojia et al. (1998) Elbetieha and Al-Hamood (1997) Al-Hamood et al. (1998) Sivakumar et al. (2014)	No effects on mating or pregnancy rates in mice in the *high* confidence RACB study (NTP, 1997) or in a *low* confidence 12-week exposure study (Elbetieha and Al-Hamood, 1997). Decreased fertility or fecundity in female rats or mice after developmental or adult exposure was reported in 3 *low* confidence studies.	• No factors noted	• Effects observed only in *low* confidence studies	⊙⊙⊙ *Indeterminate* Evidence of female reproductive effects was observed in multiple *low* confidence studies. Decreased F0 and F1 maternal body weights in a RACB study in mice (NTP, 1997) was the	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
				only notable effect in *high* confidence studies.	
MATERNAL BODY WEIGHT GAIN **High confidence:** NTP (1997) **Low confidence:** Elsaieed and Nada (2002) Junaid et al. (1995) Junaid et al. (1996b) Kanojia et al. (1998) Trivedi et al. (1989) Zheng et al. (2018)	Decreased maternal body weight was reported in 6 out of 7 studies, including F0 and F1 animals in the *high* confidence RACB study. In *low* confidence studies, decreased maternal body weights during pregnancy were concurrent with decreased fetal survival and/or fetal body weight, and authors did not adjust for gravid uterine weight to distinguish between maternal and fetal effects.	• *High* confidence study • Consistency • Dose-response gradient	• *Low* confidence studies did not adjust for gravid uterine weight		
GESTATION LENGTH **High confidence:** NTP (1997)	No effects on cumulative days to litter (F0 dams) or gestation length (F1 dams) in a *high* confidence RACB study in mice.	• *High* confidence study	• No factors noted		
HORMONES **Low confidence:** Banu et al. (2008) Banu et al. (2016) Stanley et al. (2013) Stanley et al. (2014) Samuel et al. (2012a)	Decreased serum estrogen, testosterone, and progesterone and increased FSH and LH in F1 rats in five *low* confidence studies from a single laboratory group. Decreased prolactin and growth	• No factors noted	• *Low* confidence studies, all from one research group		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	hormone also noted in one of these studies.				
ESTROUS CYCLICITY **High confidence:** NTP (1997) **Low confidence:** Kanojia et al. (1998) Murthy et al. (1996) Banu et al. (2008) Samuel et al. (2012a) Thompson et al. (2020)	No notable effects on F1 estrous cyclicity in the *high* confidence RACB study in mice. Increased estrous cycle duration in four *low* confidence studies in rats or mice exposure during development or as adults.	• No factors noted	• Effects observed only in *low* confidence studies.		
TIMING OF PUBERTY **Low confidence:** Al-Hamood et al. (1998) Banu et al. (2008) Stanley et al. (2014) Samuel et al. (2012a)	Increase in the age at pubertal onset (vaginal opening) was reported in F1 female rats or mice in four *low* confidence studies.	• No factors noted	• Effects observed only in *low* confidence studies		
ORGAN WEIGHT **High confidence:** NTP (1997) Thompson et al. (2020) **Low confidence:** Elbetieha and Al-Hamood (1997) Al-Hamood et al. (1998) Samuel et al. (2012a)	Increased relative ovary weight and decreased absolute ovary and uterus weight in 2 *low* confidence studies. Otherwise, no effects were observed.	• No factors noted	• Effects observed only in *low* confidence studies • May be secondary to decreased body weight		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
OOCYTES AND OVARIAN HISTOPATHOLOGY **High confidence:** NTP (1996a) NTP (1996b) NTP (1997) NTP (2007) Thompson et al. (2020) **Low confidence:** Kanojia et al. (1998) Murthy et al. (1996) Banu et al. (2008) Banu et al. (2015) Banu et al. (2016) Sivakumar et al. (2014) Stanley et al. (2013) Stanley et al. (2014) Samuel et al. (2012a)	No gross or microscopic changes in the ovary across 5 *high* confidence studies. Decreased corpora lutea and decreased follicle numbers and ova following superovulation in *low* confidence studies. Degenerative effects on the ovary including accelerated breakdown of germ cell nests, follicular atresia, stunted or arrested follicle development, and decreased follicle counts across 7 *low* confidence studies from a single laboratory group.	• No factors noted	• Effects observed only in *low* confidence studies, mostly from one research group		
OTHER HISTOPATHOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM **High confidence:** NTP (1996a) NTP (1996b) NTP (1997) NTP (2007) **Medium confidence:** Thompson et al. (2020)	No gross or microscopic changes were observed in the vagina, cervix, uterus, and/or clitoral gland across 5 *high* or *medium* confidence studies.	• *High* confidence studies	• No factors noted		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Mechanistic evidence					
Biological events or pathways	**Summary of key findings and interpretations**			**Judgments and rationale**	
Altered steroidogenesis	*Interpretation:* Cr(VI) alters steroidogenesis in vivo and in vitro. *Key findings:* • Decreased estrogen, testosterone, and progesterone and increased FSH and LH in animals in F1 rats following gestational exposure (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a; Banu et al., 2008). • Decreased ovarian expression of gonadotropin receptors and/or steroidogenic genes in F1 rats (Banu et al., 2016; Stanley et al., 2013) and in cultured rat granulosa cells (Stanley et al., 2013; Stanley et al., 2011; Banu et al., 2008). • Upregulation of genes involved in metabolic clearance of estradiol in F1 rats (Banu et al., 2016).			Observations of altered hormone signaling, oxidative stress, apoptosis, and effects on the ovarian extracellular matrix. Oxidative stress was concurrent with apical outcomes in some animal studies. Effects on maternal body weight gain, follicular atresia, pubertal onset, estrous cyclicity, and hormones were mitigated by cotreatment of antioxidants.	
Oxidative stress	*Interpretation:* In vivo and in vitro evidence of Cr(VI)-induced oxidative stress in female reproductive tissues concurrent with apical measurements of female reproductive toxicity. *Key findings:* • Decreased antioxidant activity or expression in the ovary was observed in F1 rats (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a), in orally exposed adult mice (Rao et al., 2009), in the rat uterus following i.p. injection (Marouani et al., 2015b), and in cultured rat granulosa and theca cells (Stanley et al., 2013). • Cotreatment of with antioxidants mitigated effects on maternal body weight gain, follicular atresia, and effects on pubertal onset, estrous cyclicity, and				

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	hormone levels (Banu et al., 2016; Stanley et al., 2014; Stanley et al., 2013; Banu et al., 2008; Elsaieed and Nada, 2002).				
Apoptosis of somatic and germ cells	*Interpretation:* In vivo and in vitro evidence of Cr(VI)-induced apoptosis in female reproductive tissues. *Key findings:* • Increased TUNEL assay staining, increased expression of pro-apoptotic markers, decreased expression of anti-apoptotic markers, and/or decreased expression of other signaling molecules that regulate cell survival reported in ovarian tissue of F1 rats (Banu et al., 2016; Banu et al., 2015; Sivakumar et al., 2014; Stanley et al., 2014; Stanley et al., 2013). Similar findings reported in adult female rats following i.p. injection (Marouani et al., 2015b). • In vitro evidence of cell cycle arrest in cultured rat granulosa cells (Stanley et al., 2011).				
Ovarian extracellular matrix	*Interpretation:* In vivo evidence that Cr(VI) induces premature ovarian failure by altering the extracellular matrix. *Key findings:* • Ovarian expression of the metalloenzyme X-propyl aminopeptidase was increased in F1 rats during late gestation and increased during early postnatal life and was inversely proportional to ovarian collagen expression (Banu et al., 2015).				

3.2.9. Developmental effects

Developmental toxicity encompasses effects that occur following pre- or postnatal exposure of the developing organism. The major categories of developmental toxicity discussed in this section are changes in survival, growth, structural alterations, and effects on the placenta. Functional effects on specific organ systems following developmental exposures are considered in their respective sections (e.g., "Male reproductive effects" and "Female reproductive effects" sections) and are also summarized here. These endpoints are considered relevant for developmental toxicity risk assessment per U.S. EPA guidelines (U.S. EPA, 1991).

This section considers both indirect (maternal or paternal) and direct routes of exposure to the developing organism. As noted previously, it is frequently difficult to determine whether effects on the fetus are in response to or separate from maternal toxicity in studies that report both, so the fetal endpoints described in this section should be considered in conjunction with the maternal endpoints described in the "Female reproductive effects" section. Developmental effects produced at doses that cause minimal maternal toxicity are still considered to represent developmental toxicity and should not be discounted as maternal toxicity (U.S. EPA, 1991). Less is known about the potential impact of paternal exposures prior to conception, but it is thought that offspring development can be affected by genetic or epigenetic changes in sperm or by direct exposure to toxicant residues in the seminal fluid.

3.2.9.1. *Human Evidence*

Study evaluation summary

Table 3-45 summarizes the eight human epidemiology studies (seven publications) considered in the evaluation of the developmental effects of Cr(VI). Two studies were excluded due to critical deficiencies in one or more domains (Xia et al., 2016; Quansah and Jaakkola, 2009). Of the six included studies, three studies (four publications) from the same research group examined male-mediated effects on offspring, specifically resulting from paternal occupational exposures to Cr(VI) from stainless-steel welding (Hjollund et al., 2005; Hjollund et al., 2000; Hjollund et al., 1995; JP et al., 1992). Exposure was measured in these studies using questionnaires. Participants were asked about their past and current welding experiences including type of metal (stainless or mild steel), welding methods, timing of welding exposures (years welding), and safety precautions used (ventilation). In each study, exposure was analyzed as stainless-steel welding/mild steel welding, and no welding. The questionnaires were not validated, and thus all the studies were evaluated as *low* confidence due to concerns in the exposure measurement domain. One study (Hjollund et al., 2000) was rated higher confidence across the other study evaluation domains and is considered to be likely the least biased and most sensitive among these *low* confidence studies. Spontaneous abortion was examined in all three, while one study (JP et al., 1992) also examined preterm birth, fetal growth, infant death within one year of birth, and congenital malformations. One general population pregnancy cohort (Peng et al., 2018) was available that examined fetal growth markers

but was limited due to exposure measurement of total chromium in urine with no additional information on occupational exposure or air sampling. In addition, two ecologic studies were available that examined associations based on proximity to a specific contaminated site (kilometers from center of polluted area in Eizaguirre-García et al. (2000), primarily affected town vs. rest of county in Remy et al. (2017)). The developmental effects examined in these studies included spontaneous abortion, early pregnancy loss (not defined), pregnancy complications, and infant health (Remy et al., 2017) and congenital malformations/anomalies (Remy et al., 2017; Eizaguirre-García et al., 2000).

Table 3-45. Summary of human studies for Cr(VI) developmental effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive data graphic for rating rationales.

Author (year)	Industry	Location	Study Design	Spontaneous abortion	Preterm birth	Fetal growth	Other (infant death, congenital malformations)
Hjollund et al. (1995), JP et al. (1992)[b]	SS Welding	Denmark	Cohort (occupational)	L	L	L	L
Hjollund et al. (2000)	SS Welding	Denmark	Cohort (occupational)	L	–	–	–
Hjollund et al. (2005)	SS Welding	Denmark	Retrospective cohort	L	–	–	–
Eizaguirre-García et al. (2000)	General population	Scotland	Ecologic	–	–	–	L
Peng et al. (2018)	General population	China	Pregnancy cohort	–	–	L	–
Remy et al. (2017)	General population	U.S.	Ecologic	L	L	–	L

SS = Stainless Steel.

[a]In addition to these included studies, two additional studies reported developmental outcomes that met PECO criteria but were found to be *uninformative* at the study evaluation stage: Quansah and Jaakkola (2009) and Xia et al. (2016).

[b]One study was described in two publications (Hjollund et al., 1995; JP et al., 1992) that reported different but overlapping subsamples. Results from both are described in the text but their results are not considered independent of each other.

Synthesis of evidence in humans

Spontaneous abortion

Four studies examined associations between spontaneous abortion and Cr(VI) exposure. Spontaneous abortion is pregnancy loss occurring before approximately 28 weeks gestation and can be subdivided into early loss (loss before pregnancy is recognized) and clinical loss (loss after 5 weeks gestation). Methods of spontaneous abortion ascertainment can vary in their ability to

identify early losses. When early losses are not detected, there is potential for bias if a true association with the exposure exists. This can even result in an apparent protective effect. In the four available studies, one was designed to ascertain early losses. Hjollund et al. (2000) used daily urine samples to identify pregnancy and early losses, which is the ideal approach. Hjollund et al. (2005) used registry data from the Danish In Vitro Fertilization Register, which includes information on clinical pregnancy identification. While this approach is not as sensitive as daily urine samples, it is likely that pregnancies were identified early in this population. The other two studies (Remy et al., 2017; Hjollund et al., 1995) identified spontaneous abortions based on hospital discharge data, which would be limited to clinical losses, and only those in women who sought medical attention.

Hjollund et al. (2000) reported a statistically significant increased risk of spontaneous abortion with paternal stainless-steel welding (RR = 3.5, 95% CI: 1.3–9.1), which was specific to this exposure group (i.e., no increase was observed with mild steel welding exposure). Conversely, Hjollund et al. (2005) and Hjollund et al. (1995) reported inverse associations (statistically significant in Hjollund et al. (2005)), although a different analysis of the population in the latter study (JP et al., 1992) reported a positive association (OR = 1.9, 95% CI: 1.1–3.2). However, in this latter analysis, spontaneous abortion was based on registry data providing the number of spontaneous abortions preceding each birth recorded in the national registry, and this measure was considered to be less sensitive than measures in other studies. In addition, in JP et al. (1992), there were similarly higher odds for induced abortion (OR = 2.1, 95% CI: 1.2–3.4), which increases uncertainty about the reliability of the estimate since there is no plausibility for Cr(VI) to influence induced abortions. A *low* confidence ecologic study (Remy et al., 2017) also reported higher relative risk of spontaneous abortion with higher exposure (RR 1.80, 95% CI: 1.20, 2.68). Overall, there is some indication that Cr(VI) exposure is associated with spontaneous abortion, most notably in Hjollund et al. (2000), which had the highest quality outcome ascertainment methods able to ascertain early losses. It is possible that the inverse associations observed in Hjollund et al. (1995) were due to early losses missed by their outcome ascertainment methods, but there is not adequate data to assess this. However, given the small number of studies and the limited nature of the evidence there is considerable uncertainty.

Fetal growth, preterm birth, and infant death:

Three studies (Peng et al., 2018; Remy et al., 2017; JP et al., 1992) examined associations with fetal growth outcomes, though in Remy et al. (2017) the association was reported for a combination of outcomes that also included preterm birth. Peng et al. (2018) examined birth weight, length, and ponderal index, as well as fetal ultrasound measurements of head and abdominal circumference and femur length in all three trimesters. There were statistically significant decreases in ponderal index with increased exposure, and non-statistically significant decreases in birth weight and fetal head and abdominal circumference and femur length (in the third trimester only). JP et al. (1992) reported no association with low birthweight. Remy et al.

(2017) reported higher relative risk for preterm birth, low birthweight, and small for gestational age combined (RR 1.14, 95% CI: 1.05, 1.25). Thus, there is some indication of fetal growth restriction with Cr(VI) exposure, but there is considerable uncertainty as the exposure in Peng et al. (2018) was total chromium and Remy et al. (2017) also included preterm birth, both of which reduce the interpretability of the findings.

In addition, JP et al. (1992) reported on preterm birth and infant death within the first year. They reported a non-statistically significant association between higher Cr(VI) exposure levels and increased odds of preterm birth (OR = 1.3, 95% CI: 0.9–1.9). No association was observed for infant mortality, but the lack of association could be due at least in part to poor sensitivity as above. In addition to the preterm birth results already discussed, Remy et al. (2017) reported higher relative risk for perinatal jaundice (RR 1.13, 95% CI: 1.06, 1.20) and some infant health conditions (infectious/parasitic, nervous system). While both studies reported associations with preterm birth, this was analyzed in a combined outcome in Remy et al. (2017), which again makes it difficult to interpret. The other outcomes were observed in a single *low* confidence study.

Congenital malformations

Three studies examined the association between Cr(VI) exposure and congenital malformations (Remy et al., 2017; Eizaguirre-García et al., 2000; JP et al., 1992). In JP et al. (1992), there was no association between paternal occupational exposure and congenital malformations. In Eizaguirre-García et al. (2000), risk of congenital malformations was lowest in areas closest to the center of the polluted area. In Remy et al. (2017), there was higher relative risk of eye, ear, face, neck, and cleft anomalies in the higher exposed geographic area (RR 1.19, 95% CI: 0.91, 1.56), but this was only observed in one of the two time periods studied. No increase in genitourinary anomalies was observed. Overall, there is limited evidence of an association between congenital malformations and Cr(VI) exposure. However, all of the available studies had serious limitations which limits interpretation of their results.

In summary, there are some indications of an association between Cr(VI exposure and spontaneous abortion, fetal growth, preterm birth, and congenital malformations, but the evidence is limited in quality and quantity.

3.2.9.2. *Animal Evidence*

Study evaluation summary

Table 3-46 summarizes the animal toxicology studies considered in the evaluation of the developmental effects of Cr(VI). These consist of one continuous breeding study using NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (NTP, 1997); four studies that evaluated effects in F1 offspring following maternal-only exposure (Kanojia et al., 1998; Elbetieha and Al-Hamood, 1997) or paternal-only exposure (Marat et al., 2018; Al-Hamood et al., 1998; Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997) prior to mating; and thirteen studies that

evaluated F1 offspring from dams that were exposed during gestation (Zheng et al., 2018; Arshad et al., 2017; Banu et al., 2017b; Banu et al., 2017c; Kumar et al., 2017; Shobana et al., 2017; Samuel et al., 2012a; Bataineh et al., 2007; De Flora et al., 2006; Elsaieed and Nada, 2002; Junaid et al., 1996b, 1995; Trivedi et al., 1989) or lactation (Sánchez et al., 2015). All studies were oral exposures (diet, drinking water, or oral gavage), although exposure to offspring was indirect in all studies except the RACB study.

The RACB study by NTP (1997) and the gestational exposure study by Zheng et al. (2018) were well-reported and well-designed to evaluate effects in developing animals and therefore were rated as *high* confidence for all reported outcomes. The studies by De Flora et al. (2006) and Shobana et al. (2017) had minor concerns raised during study evaluation and were rated *medium* confidence. The remaining studies had reporting limitations and other substantial concerns and were rated as *low* confidence across all outcomes. Endpoint-specific concerns are discussed in the respective sections below. Three of the *low* confidence studies (Al-Hamood et al., 1998; Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997) exposed animals to high concentrations (350–1770 mg/L) of Cr(VI) in drinking water, which was considered a potential confounding variable as it is not possible to determine whether developmental effects may have been exacerbated by reduced water consumption and/or systemic toxicity; for instance, drinking water concentrations of 350 mg/L Cr(VI) have been associated in rats with decreased water consumption and site of contact toxicity (80 and 100% incidence of ulcers in the glandular stomach of males and females, respectively) (NTP, 2007). There were concerns about scientific integrity for two groups of authors[49] (Banu et al., 2017b; Banu et al., 2017a; Kumar et al., 2017; Samuel et al., 2012a; Kanojia et al., 1998; Junaid et al., 1996b, 1995), which reduces confidence in these studies but does not necessarily discount the results.

[49]Four studies demonstrating self-plagiarism—i.e., publication of identical data presented as separate and unique experiments—were considered *critically deficient* and were excluded from the assessment. Specifically, 1) identical data were presented for rats by Kanojia et al. (1996) and for mice by Junaid et al. (1996a), despite these being presented as separate studies in different species; and 2) subsets of the data presented by Samuel et al. (2012b; 2011) were identical to that in an earlier publication by this laboratory group (Banu et al., 2008). Other studies by the same groups of authors, listed in the text above, were included in the assessment but considered *low* confidence.

Table 3-46. Summary of included studies for Cr(VI) developmental effects and overall confidence classification [high (H), medium (M), low (L)] by outcome.[a]
Click to see interactive graphic for ratings rationale.

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Survival	Growth	Structural	Placenta	Functional effects
NTP (1997)	Mice (BALBC)	Reproductive Assessment by Continuous Breeding (F0 to F2)	Diet	H	H	-	-	H
Al-Hamood et al. (1998)	Mice (BALBC)	F1 males or females exposed GD 12–PND 20 and mated with untreated animals	Drinking water	L	-	-	-	L
Banu et al. (2008)	Rat (Wistar)	F1 females; PND 1–21	Drinking water	-	-	-	-	L
Banu et al. (2015)	Rat (Sprague-Dawley)	F1 females; GD 9.5–14.5	Drinking water	-	-	-	-	L
Banu et al. (2016)	Rat (Sprague-Dawley)	F1 females; PND 1–21	Drinking water	-	-	-	-	L
Banu et al. (2017a)	Rat (Sprague-Dawley)	GD 9.5–14.5	Drinking water	-	L	-	-	-
Banu et al. (2017b)	Rat (Sprague-Dawley)	GD 9.5–14.5	Drinking water	-	-	-	L	-
Bataineh et al. (1997)	Rat (Sprague-Dawley)	F0 males exposed 12 weeks prior to mating with untreated females	Drinking water	L	-	-	-	-
De Flora et al. (2006)	Mice (Swiss albino)	"Duration of pregnancy"–GD 18	Drinking water	M	M	-	-	-
Elbetieha and Al-Hamood (1997)	Mice (Swiss)	F0 males or females exposed 12 weeks prior to mating with untreated animals	Drinking water	L	-	-	-	-
Elsaieed and Nada (2002)	Rat (Wistar)	GD 6–15	Drinking water	L	L	L	L	-
Junaid et al. (1995)	Mice (Swiss albino)	GD 14–19	Drinking water	L	L	L	L	-
Junaid et al. (1996b)	Mice (Swiss albino)	GD 6–14	Drinking water	L	L	L	L	-
Kanojia et al. (1998)	Rat (Druckrey)	F0 females exposed 3 months prior to mating with untreated males	Drinking water	L	L	L	L	-
Kumar et al. (2017)	Rat (Wistar)	GD 9–14	Drinking water	-	L	-	-	L
Samuel et al. (2012a)	Rat (Wistar)	*Study 1*: GD 9–21 *Study 2*: GD 9–PND 65	Drinking water	L	L	-	-	L

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Survival	Growth	Structural	Placenta	Functional effects
Shobana et al. (2017)	Rat (Wistar)	GD 9–14	Drinking water	-	-	-	-	M
Sivakumar et al. (2014)	Rat (strain not reported)	F0 dams; GD 9.5–14.5	Drinking water	-	-	-	-	L
Stanley et al. (2013)	Rat (Sprague-Dawley)	F1 females; PND 1–21	Drinking water	-	-	-	-	L
Stanley et al. (2014)	Rat (Sprague-Dawley)	F1 females; PND 1–21	Drinking water	-	-	-	-	L
Trivedi et al. (1989)	Mice (albino)	GD 0–19	Drinking water	L	L	L	L	-
Arshad et al. (2017)	Mice (Swiss-Webster)	GD 6	Gavage	L	L	L	-	-
Bataineh et al. (2007)	Rat (Sprague-Dawley)	GD 1–3 or 4–6	Gavage	L	-	-	-	-
Marat et al. (2018)	Rat (white outbred)	Adult males; 60 days	Gavage	L	-	-	-	-
Sánchez et al. (2015)	Rat (Wistar)	PND 4–19	Gavage	-	-	L	-	-
Zheng et al. (2018)	Rat (Sprague-Dawley)	GD 12–21.5	Gavage	H	H	-	-	H

GD = gestation day; PND = postnatal day

[a]In addition to these included studies, there were seven animal toxicology studies reporting female reproductive effects that met PECO criteria but were found to be *uninformative* at the study evaluation stage: Junaid et al. (1996a), Kanojia et al. (1996), Soudani et al. (2011b), Soudani et al. (2011a), Soudani et al. (2013), Zahid et al. (1990), and Borneff et al. (1968).

Synthesis of evidence in animals[50] [51]

Fetal and postnatal survival

Decreased offspring survival was observed only in *low* confidence studies. Statistically significant effects occurred at the same dose or lower compared to decreased maternal body weight gain or clinical signs of maternal toxicity within a subset of studies that reported both maternal and fetal endpoints (Elsaieed and Nada, 2002; Kanojia et al., 1998; Junaid et al., 1996b, 1995; Trivedi et al., 1989). Other *low* confidence studies provided little or no data on maternal toxicity, so the relative sensitivity of maternal and offspring effects could not be compared in those cases.

[50]Data are available in HAWC for NTP (1997) here.

[51]For many of the oral studies presented here, it was not possible to estimate an average daily mg/kg dose due to lack of reporting. To estimate an average daily dose, paired records of body weight and daily intake of test article are required. This is particularly important for Cr(VI) reproductive and developmental studies, because rapid changes in maternal body weight are expected during pregnancy, and Cr(VI) affects palatability (which affects both Cr(VI) intake rate and body weight). Doses of Cr(VI) are presented where possible, however many cross-study comparisons are done on the basis of mg/L Cr(VI) in drinking water.

In the *high* confidence RACB study in mice (NTP, 1997) there was no effect on the number of live pups per litter or proportion of pups born alive across the F1 and F2 litters at dietary doses up to 30.3 mg/kg-day Cr(VI) (F0 parental animals) and 37.1 mg/kg-day Cr(VI) (F1 parental animals), and no effects on survival of F1 from birth until weaning at PND 21. The *high* confidence gestational exposure study by Zheng et al. (2018) also reported no effects on rat pup numbers or sex ratio (% male pups) following maternal exposure at doses up to 12 mg/kg-d Cr(VI) via oral gavage from GD 12–21. The *medium* confidence gestational exposure study by De Flora et al. (2006) reported no effect on the number of fetuses at GD 18 following maternal exposure to 5 or 10 mg/L Cr(VI) in drinking water throughout the duration of pregnancy.

In contrast to the findings in *high* and *medium* confidence studies, all *low* confidence studies that exposed dams to Cr(VI) during pregnancy reported increased pre- or post-implantation loss. Rat dams dosed with 25 mg/kg-day potassium dichromate via oral gavage from GD 1–3 had no implantations Bataineh et al. (2007); and a dose-related increase in pre-implantation loss was observed in mice exposed from GD 0–19, reaching statistical significance at 177 mg/L Cr(VI) (Trivedi et al., 1989). Statistically significant increases in pre-implantation loss were also reported in rats exposed to 50 mg/L potassium dichromate in drinking water from GD 6–15 (Elsaieed and Nada, 2002), and a dose-related decrease in implantation index (number of implantation sites / number of corpora lutea) was reported in rats exposed to 50–400 mg/L Cr(VI) from GD 9–21 (Samuel et al., 2012a); however, these exposures began around or after the time of implantation in rats (generally GD 6) and therefore effects may not have been related to treatment (U.S. EPA, 1991). Statistically significant dose-related increases in post-implantation loss (resorptions or dead fetuses) were observed in mice following exposure from GD 0–19 (Trivedi et al., 1989), GD 6–14 (Junaid et al., 1996b), and GD 14–19 (Junaid et al., 1995), reaching statistical significance at 88 or 177 mg/L Cr(VI). In studies that tested a single dose level, post-implantation loss was increased in rats following exposure to a maternal dose of 50 mg/L Cr(VI) in drinking water from GD 6–15 (Elsaieed and Nada, 2002), in rats given a maternal dose of 8.8 mg/kg-day Cr(VI) via oral gavage from GD 4–6 (Bataineh et al., 2007), and in mice given a maternal dose of 3.9–16 mg/kg Cr(VI) via oral gavage on GD 6 (Arshad et al., 2017). The studies by Arshad et al. (2017) and Samuel et al. (2012a) presented results in terms of the number of individual fetuses affected without indication of means or variance across litters, so there is greater uncertainty in the results of these studies.

Three *low* confidence studies reported decreased fetal survival when maternal animals were exposed to Cr(VI) prior to mating. Kanojia et al. (1998) exposed rat dams to Cr(VI) via drinking water for 3 months prior to mating with unexposed males and reported a dose-related 2- to 3.1-fold increase in pre-implantation loss and a 2.2- to 4.2-fold increase in post-implantation loss, reaching statistical significance at 88 mg/L Cr(VI). Elbetieha and Al-Hamood (1997) exposed adult F0 female mice to Cr(VI) in drinking water for 12 weeks prior to mating with unexposed males, and reported a 17–18% decrease in implantations, ~5–6 fold increase in the number of mice with resorptions, and a 25–32% decrease in viable fetuses, all of which were statistically significant at

both of the tested doses [707 and 1,768 mg/L Cr(VI)]. (Al-Hamood et al., 1998) exposed F1 female mice to maternal doses of 353 mg/L Cr(VI) in drinking water during development (from GD 12–PND 20) and then mated these animals with unexposed males as adults, and reported a statistically significant 12% decrease in implantations and 14% decrease in viable fetuses.

Male-mediated decreases in fetal survival were observed in two *low* confidence paternal-only exposure studies. Elbetieha and Al-Hamood (1997) reported a statistically significant 16–23% decrease in implantations and viable fetuses when adult F0 male mice were exposed to 707 or 1,414 mg/L Cr(VI) in drinking water for 12 weeks prior to mating with untreated females; these effects were not observed at the 353 or 1,768 mg/L dose levels, although some resorptions or dead fetuses were noted. Marat et al. (2018) exposed adult F0 male rats to 0.353 mg/kg-day Cr(VI) via oral gavage for 60 days prior to mating with untreated females and reported a 1.8-fold increase in pre-implantation mortality, an 8.9-fold increase in post-implantation mortality, and a dominant lethal mutation frequency of 0.665. There were no effects on the number of implantation sites and viable fetuses in two other *low* confidence paternal exposure studies, both of which exposed parental males to a dose level of 353 mg/L Cr(VI) in drinking water during development (Al-Hamood et al., 1998) or as adults (Bataineh et al., 1997) and mated with unexposed females.

Fetal and postnatal growth

Decreased fetal or postnatal growth were observed to some extent in almost all studies that evaluated these outcomes. Statistically significant effects occurred at the same dose or lower compared to decreased maternal body weight gain or clinical signs of toxicity within a subset of studies that reported both maternal and fetal endpoints (Elsaieed and Nada, 2002; Kanojia et al., 1998; NTP, 1997; Junaid et al., 1996b, 1995; Trivedi et al., 1989). Other *low* confidence studies provided little or no data on maternal toxicity, so the relative sensitivity of maternal and offspring effects could not be compared in those cases.

In the *high* confidence RACB study in mice, mean F1 male and female pup body weights in the highest dose group [F0 dietary exposure of 30.3 mg/kg-day Cr(VI)] were similar to controls at birth but were 9–15% lower than controls at PNDs 14 and 21, although this effect was not statistically significant[52]. By PND 74 ± 10, the effect on F1 body weights was statistically significant; mean F1 male and female body weights in the highest dose group [37.1 mg/kg-day Cr(VI)] were decreased by 9% compared to controls, and F1 females in the second highest dose group [16.1 mg/kg-day Cr(VI)] were decreased by 4% compared to controls (NTP, 1997). Food consumption was increased in the treated animals compared to controls, so the decrease in growth does not seem to be attributable to palatability or changes in feed consumption. There was a statistically significant 11% decrease in F2 female pup birth weights at 37.1 mg/kg-day Cr(VI), although pup body weights in this group were not statistically significantly lower than controls

[52]Data are available for males (PND14 and PND21) and females (PND14 and PND21).

when adjusted for litter size. Otherwise, there were no effects on F2 pup birth weights, and F2 animals were not monitored further.

The remaining studies that observed decreased F1 growth were considered *low* confidence. Kanojia et al. (1998) exposed rat dams via drinking water for 3 months prior to mating and reported a dose-related 21–36% decrease in fetal body weight, reaching statistical significance at 88 mg/L Cr(VI). In drinking water studies that exposed pregnant dams, fetal body weights were decreased in a dose-related manner compared to controls by 18–47% (Junaid et al., 1995), 3–19% (Junaid et al., 1996b), and 32–44% (Trivedi et al., 1989) following exposure from GDs 14–19, 6–14, and 0–19, respectively, reaching statistical significance at 88 or 177 mg/L Cr(VI). Two studies that exposed pregnant dams to 50 mg/L Cr(VI) observed that fetal body weights were statistically significantly decreased compared to controls by 33% following maternal exposure from GD 6–14 (Elsaieed and Nada, 2002) and by 31% following maternal exposure from GD 9.5–14.5 (Banu et al., 2017a)[53]. One study that exposed pregnant mice on GD 6 via oral gavage reported that fetal body weights were decreased by 17–27% compared to controls, reaching statistical significance at 22 ug/g potassium dichromate (Arshad et al., 2017). Three of the gestational exposure studies also reported decreased crown-rump length (Arshad et al., 2017; Junaid et al., 1995; Trivedi et al., 1989), and the study by Arshad et al. (2017) reported decreased morphometric parameters including head and eye circumference, and fore limb, hind limb, and tail length. In two studies that assessed postnatal growth, Kumar et al. (2017) reported a dose-related statistically significant 11–20% decrease in body weight at PND 120 in F1 male rats from dams that had been exposed to 35.3 or 70.7 mg/L Cr(VI) in drinking water from GD 9–14, and Samuel et al. (2012a) reported a statistically significant 33–41% decrease in body weights on PNDs 3, 7, 18, 45, and 65 in F1 female rats that had been continuously exposed to 200 mg/L Cr(VI) in drinking water from GD 9–PND 65[54]. The studies by Banu et al. (2017a), Kumar et al. (2017), and Samuel et al. (2012a) reported body weights as the mean of individual offspring without accounting for litter effects, and it was not clear whether results in the studies by Elsaieed and Nada (2002) or Arshad et al. (2017) were litter means or the means of individual animals; this affects interpretation of the results in these studies, as failure to consider litter effects has the potential to overestimate statistical significance (Haseman et al., 2001).

Three studies reported no effect on F1 growth. The *high* confidence study in rats by Zheng et al. (2018) reported no change in newborn pup body weight following maternal exposure at doses up to 12 mg/kg-d Cr(VI) via oral gavage from GD 12–21. The *medium* confidence study in mice by De Flora et al. (2006) reported no change in fetal body weight at GD 18 following maternal

[53]Fetal body weights in Banu et al. (2017a) were reported graphically, but were estimated using WebPlotDigitizer to be 2.64 ± 0.01 g in the control group and 1.82 ± 0.14 g in the Cr(VI) exposure group.
[54]F1 body weights in Kumar et al. (2017) and Samuel et al. (2012a) were reported graphically and were estimated using WebPlotDigitizer. The difference in body weights between control and Cr(VI)-exposed animals on PND 3 in the study by Samuel et al. (2012a) could not be estimated using WebPlotDigitizer due to the scale of the figure, so the values shown are for PNDs 7, 18, 45, and 65.

exposure to 5 or 10 mg/l Cr(VI) in drinking water throughout the duration of pregnancy. The *low confidence* study by Al-Hamood et al. (1998) reported no effects on male or female body weight at PND 50 in F1 mice that had been exposed to maternal doses of 353 mg/L Cr(VI) in drinking water from GD 12–PND 20.

Structural alterations

A dose-related increase in structural alterations was reported in all studies that evaluated these outcomes in fetuses or early postnatal animals, which consisted of *low* confidence studies. Statistically significant effects occurred at the same dose or lower compared to decreased maternal body weight gain or clinical signs of toxicity within a subset of studies that reported both maternal and fetal endpoints (Elsaieed and Nada, 2002; Kanojia et al., 1998; Junaid et al., 1996b, 1995; Trivedi et al., 1989), whereas the other two studies did not provide data on maternal toxicity. Within studies, reduced ossification occurred at doses concurrent with decreased fetal growth (body weight or morphometric parameters) and was mostly observed in bones that undergo rapid ossification at the end of gestation (e.g., parietals, interparietals, caudal, frontals). This may indicate that the delay in ossification is indicative of a generalized growth delay (Carney and Kimmel, 2007).

Four *low* confidence studies by the same research group evaluated fetuses at GD 19. Reduced skeletal ossification was observed when F0 rat dams were exposed to potassium dichromate in drinking water for 3 months prior to mating (Kanojia et al., 1998) and when F0 mouse dams were exposed to potassium dichromate in drinking water from GD 0–19 (Trivedi et al., 1989), GD 6–14 (Junaid et al., 1996b), or GD 14–19 (Junaid et al., 1995). Skeletal effects across these studies reached statistical significance at levels as low as 88 mg/L Cr(VI). Trivedi et al. (1989) also reported that fetuses had decreased number of ribs, which reached statistical significance at 177 mg/L Cr(VI). In addition to skeletal effects, these four studies each reported the same gross abnormalities (drooping wrist, subdermal hemorrhagic patches, kinking tail, short tail) and reported that the exposed animals did not have any visceral alterations. Trivedi et al. (1989) also reported "enlarged gap between fingers."

The remaining *low* confidence studies that evaluated fetal structural alterations have greater uncertainty due to incomplete reporting of results. Elsaieed and Nada (2002) reported a statistically significant increase in skeletal and visceral abnormalities in fetuses from F0 rat dams that were exposed to 50 mg/L Cr(VI) in drinking water from GD 6–15 and sacrificed on the day before delivery, and noted that some animals had incomplete ossification of the skull bone and increased renal dilation; however, data were reported as the average total skeletal and visceral abnormalities per litter with no quantitative incidence data provided for specific alterations. Arshad et al. (2017) reported numerous skeletal and visceral abnormalities in mouse fetuses from dams that were dosed on GD 6 with 3.8–16 mg/kg Cr(VI) via oral gavage and sacrificed on GD 18, including reduced skeletal ossification; however, most of these abnormalities were described qualitatively with no information provided on relative incidence. Quantitative incidence data was

provided for some abnormalities (anophthalmia, limb hyperextension, limb hyperflexion, limb malrotation, limb micromelia, and spina bifida) but was reported as the total number of individual fetuses affected without indication of potential litter effects. Sánchez et al. (2015) evaluated periodontal bone development in rats dosed with 4.4 mg/kg-day Cr(VI) via oral gavage from PND 4–15 and observed that the exposed animals had statistically significant decreases in dental alveolar bone volume, periodontal width, and degree of tooth eruption compared to controls, as well as a decrease in dental alveolar surfaces covered in osteoblasts or osteoclasts (which are involved in bone formation and remodeling) and an increase in resting bone surfaces covered in bone lining cells. The authors did not indicate whether offspring growth was affected in this study, which is a factor that is would be expected to affect tooth eruption; and reported data as the means of individual pups, which has the potential to overestimate statistical significance (Haseman et al., 2001).

Effects on the placenta

Effects on the placenta were evaluated in several *low* confidence studies that exposed dams to Cr(VI) prior to or during gestation. Placental effects occurred at the same doses as decreased maternal body weight gain in the studies that provided both maternal and fetal data (Elsaieed and Nada, 2002; Kanojia et al., 1998; Junaid et al., 1995), although some studies reported decreased maternal body weight gain but no effect on placenta weights.

Two *low* confidence studies evaluated placental histopathology. In rat dams exposed to 50 mg/L potassium dichromate in drinking water from GD 6–15, Elsaieed and Nada (2002) reported histologic lesions in the placenta including necrosis in the chorionic villi and focal extravasation of red blood cells in the decidua basalis. Banu et al. (2017b) reported histologic effects including increased hypertrophy and hemorrhagic lesions in the basal zone in rat dams exposed to 17.7 mg/L Cr(VI) in drinking water from GD 9.5–14.5. Neither of these studies provided quantitative data on the incidence or severity of these lesions, so interpretation of these findings is limited.

Changes in placenta weight were also observed in *low* confidence studies, although the direction of effect was inconsistent across studies. Rat dams exposed to potassium dichromate in drinking water for 3 months prior to mating had statistically significantly decreased placenta weights in the 177 and 265 mg/L Cr(VI) dose groups in the study by Kanojia et al. (1998), whereas a statistically significant dose-related increase in placenta weight was observed at exposure levels ≥88 mg/L Cr(VI) in mouse dams exposed from GD 14–19 in the study by Junaid et al. (1995). In other *low* confidence studies, no effects on placenta weight were observed in mouse dams exposed to levels up to 265 mg/L Cr(VI) in drinking water from GD 6–14 (Junaid et al., 1996b) or up to 177 mg/L Cr(VI) in drinking water from GD 0–19 (Trivedi et al., 1989).

Functional effects (reproductive, endocrine)

Effects on the developing reproductive system are described in the "Male reproductive effects" and "Female reproductive effects" sections and summarized briefly here. Effects on F1 male

and female fertility and histopathology were not observed in the *high* confidence RACB study (NTP, 1997) at doses up to 37.1 mg/kg-day Cr(VI) via diet, but were documented in several other studies. In F1 male rats, a nonmonotonic effect on testosterone (increased at 3 mg/kg-day, decreased at 12 mg/kg-day) and altered Leydig cell distribution were observed following maternal exposure by oral gavage from GD 12–21 in the *high* confidence study by Zheng et al. (2018). The *low* confidence study by Kumar et al. (2017) reported decreased sperm quality, histopathological changes in the testis, decreased testosterone and gonadotropins, and decreased reproductive organ weights in F1 males exposed from GD 9–14 to maternal doses of 17.7–70.7 mg/L Cr(VI) in drinking water. In F1 female rats, a series of *low* confidence studies by one laboratory group reported pathological effects on oocyte development following gestational and/or postnatal exposure to maternal doses of 8.8–70.7 mg/L Cr(VI) in drinking water, as well as decreased sex steroid hormone levels, increased gonadotropin levels, delayed puberty, and changes in estrous cyclicity (Banu et al., 2016; Banu et al., 2015; Sivakumar et al., 2014; Stanley et al., 2014; Stanley et al., 2013; Samuel et al., 2012a; Banu et al., 2008). A *low* confidence study in mice by Al-Hamood et al. (1998) likewise reported decreased pregnancy rates and delayed puberty in F1 males that had been exposed to maternal doses of 353 mg/L Cr(VI) from GD 12–PND 20. Interpretation of the *low* confidence studies is limited, due to the study design and reporting concerns discussed in "Male reproductive effects" and "Female reproductive effects" sections.

Other evidence of functional effects in developing animals comes from a *medium* confidence study that evaluated insulin signaling in F1 rats following maternal exposure to potassium dichromate in drinking water from GD 9–14 (Shobana et al., 2017). Serum insulin levels in pubertal F1 rats evaluated on PND 59 were statistically significantly increased compared to controls at maternal exposure levels ≥50 mg/L. Glucose uptake was increased in liver but decreased in skeletal muscle, and glucose oxidation was increased in both liver and skeletal muscle at 50 mg/L Cr(VI) but decreased at 100 and 200 mg/L Cr(VI). Despite these changes, there was no effect on fasting blood glucose or oral glucose tolerance in these animals.

3.2.9.3. *Mechanistic Evidence*

Studies providing mechanistic evidence on the potential developmental effects of Cr(VI) are tabulated in Appendix C.2.8 and summarized here. Together, these studies provide supporting evidence that Cr(VI) may have adverse developmental effects if it were to reach the relevant target tissues. The mechanistic studies reviewed here consisted of in vivo mechanistic data from several oral exposure studies, most of which are discussed above (Table 3-46), as well as data from intraperitoneal (i.p.) injection studies, in vitro studies in whole embryos, and in vitro studies in trophoblast or osteoblast cell lines that did not meet PECO criteria but were reviewed as informative to the mechanistic analysis. Dosing via i.p. injection is likely to result in higher tissue concentrations of Cr(VI) compared to oral exposure, since an oral first-pass effect exists due to the reduction of Cr(VI) in the low pH environment of the stomach; less than 10–20% of an ingested dose may be absorbed in the GI tract, and further reduction will occur in the liver prior to

distribution to the rest of the body (see Section 3.1 and Appendix C). Therefore, systemic effects are expected to be more likely following i.p. injection or inhalation compared to oral exposure. Effects are also expected to be more likely in in vitro embryonic studies compared to in vivo studies, since the in vitro studies incubated sperm or blastocytes directly with potassium dichromate.

Fetal genotoxicity

One study assessed genotoxicity [measured as the frequency of micronucleated (MN) polychromatic erythrocytes (PCE) in maternal bone marrow and fetal liver and peripheral blood] in mice exposed to Cr(VI) salts during gestation via i.p. injection or oral exposure (De Flora et al., 2006). Fetuses from dams dosed orally via drinking water with sodium dichromate dihydrate (5 or 10 mg/l) or potassium dichromate (10 mg/l) did not have any changes in the frequency of MN PCE compared to controls. In contrast, fetuses from dams given a single i.p. injection of 50 mg/kg potassium dichromate or sodium dichromate dihydrate on GD 17 had significantly increased frequency of MN PCE frequency in the liver and peripheral blood. The same pattern was observed in maternal bone marrow. This study suggests that Cr(VI) is genotoxic to fetuses when it reaches target tissues, although bioavailability is poor through the oral route of exposure.

In vitro evaluations of embryo development

Three studies in whole embryos provided evidence that Cr(VI) impairs embryonic development. One study incubated mouse sperm with potassium dichromate and used it to fertilize eggs from untreated mice (Yoisungnern et al., 2015). It was found that the percentage of unfertilized oocytes and embryos in the 2-cell stage increased while the percentage in the expanded and hatching blastocyst stages and total number of blastocysts were decreased, suggesting delays in embryonic development. These effects were observed at the lowest dose level (1.1 μM Cr(VI)), and differences became more pronounced with increasing doses, although higher doses also produced statistically significant decreases in sperm viability. Blastocysts in the low dose group also had a decrease in the number of trophectoderm and inner cell mass cells and decreased expression of pluripotent marker genes (*sox2*, *pou5f1*, and *klf4*), indicating impaired development of the embryo and placenta. A second study that incubated mouse blastocysts with potassium dichromate (Iijima et al., 1983) found a dose-dependent decrease in 2-layer inner cell masses after 6 days of exposure to 0.088–0.71 μM Cr(VI), but statistically significant differences in hatching, attachment and trophoblast outgrowths were not observed. Cultured embryos treated for 24 hours with 0.18–0.71μM Cr(VI) showed statistically significant decreases in allantois fusion, beating hearts, and blood islands. Decreased crown-rump length was also observed at doses of 0.35–0.71 μM Cr(VI). Additionally, a third study that collected mouse embryos at the 2-cell stage and incubated them in culture with potassium dichromate or calcium chromate reported that Cr(VI) salts inhibited blastocyst formation and hatching in a dose-dependent manner, with the high dose of potassium dichromate (7.1 μM Cr(VI)) arresting embryonic development at the 4-cell stage (Jacquet and Draye, 1982).

Mechanisms affecting bone development

Several in vitro and in vivo studies identified mechanisms that that are potentially relevant to skeletal alterations and suggested oxidative stress as an underlying mechanism. In vitro studies with immortalized rat osteoblasts show that Cr(VI) inhibits cell viability and decreases cellular activity (protein, DNA, and RNA synthesis; production of collagen fibers) and found that effects were mitigated by Vitamin C (ascorbic acid), which is an antioxidant (Ning et al., 2002; Ning and Grant, 2000, 1999).

Additionally, thyroid effects [decreased triiodothyronine (T3) and thyroxine (T4), and follicle size and increased TSH concurrent with morphology changes] were observed in adult male rats following injection with 21 µg/kg Cr(VI) and were partially prevented when animals were pretreated or cotreated with ascorbic acid (Qureshi and Mahmood, 2010). Thyroid function is important for skeletal developmental and disruption can result in delays in skeletal ossification; however, the relevance of this finding to developing animals is unclear since this study was conducted in adults.

Mechanisms affecting insulin regulation

The gestational exposure study in rats by Shobana et al. (2017), described in the section above, also provided mechanistic information relevant to insulin signaling. Insulin receptor protein expression in liver and gastrocnemius muscle was decreased, suggesting negative feedback resulting from increased insulin levels, and decreasing trends were observed in the expression of insulin receptor substrate-1 (IRS-1) and its phosphorylated form (p-IRS-1^{tyr632}) in these tissues. In liver, the expression of the downstream signaling molecule Akt was unchanged while the phosphorylated form (p-Akt^{Ser473}) increased; whereas in gastrocnemius muscle, Akt expression decreased and the effects on p-Akt^{Ser473} were nonmonotonic (increased at 50 mg/L Cr(VI) but decreased at 100 mg/L Cr(VI)). GLUT 2 was increased in liver at 50 mg/L Cr(VI) and GLUT 4 was decreased in gastrocnemius muscle at 200 mg/L, reflecting glucose uptake in these tissues. PPARγ expression in these tissues was increased, which the authors speculated may be involved in the regulation of glucose transporters.

Oxidative stress and apoptosis in the placenta

Studies in humans, rats, and human cell lines provide supporting evidence for oxidative damage and apoptosis in the placenta, as well as evidence that chromium reaches human placental tissue. Placentae collected from healthy women in the general population showed average chromium concentrations between 0.02 to 1.25 mg/L (Banu et al., 2018), although these were total chromium concentrations and it was unclear whether the women were exposed to Cr(VI) or another form of Cr. Two biomarkers of oxidative stress in the samples with the highest average chromium concentrations were statistically significantly increased over the lowest concentration group and differences were also noted in the mRNA and protein expression of some antioxidants, but there are uncertainties in the interpretation of this data; several apoptotic markers

(e.g., cytochrome C, AIF, Bax and cleaved caspase-3) were elevated in addition to anti-apoptotic markers Bcl-2 and Bcl-XL, and some results showed sexually dimorphic differences (Banu et al., 2018).

Two studies evaluated placentae in rats administered 17.7 mg/L Cr(VI) in drinking water during gestation. Banu et al. (2017c) performed immunohistochemical analysis demonstrating decreased trophoblast cell populations and decreased expression of cyclin D1 in the placentas, and found that placentas of Cr(VI)-treated dams had increased biomarkers of oxidative stress (LPO and H2O2) and decreased expression of antioxidant enzymes (SOD, Gpx, Prdx3, and Txn2). Banu et al. (2017b) reported increases in apoptosis and caspase-3 in the maternal compartment (metrial gland) and the caspase-3 independent apoptotic marker AIF in both the fetal and maternal compartments. Increases in p53 and related signaling cascade molecules were also observed.

Two studies evaluated placental cells in vitro. Banu et al. (2018) evaluated the human trophoblastic cell line BeWo and observed a dose-related decrease in the mRNA expression of antioxidant enzymes (SOD, Gpx, Prdx3 and Txn2) following dosing with 1.8–11 µM Cr(VI) for 12–24 hours. Another in vitro study by Sawicka and Długosz (2017) observed increased lipid peroxidation and decreased antioxidant enzyme activity (SOD, GST) in mitochondria isolated from human placental tissue following treatment with 0.05–1 µg/mL Cr(VI). The increase in lipid peroxidation and decrease in SOD were mitigated by cotreatment with an estradiol metabolite, 4-OHE2.

3.2.9.4. *Integration of Evidence*

Overall, the available **evidence indicates** that Cr(VI) likely causes developmental effects in humans under relevant exposure circumstances. This conclusion is primarily based on the observation of decreased offspring growth across most animal studies, as evidenced by decreased fetal or postnatal body weights and decreased skeletal ossification. Other outcomes in animal studies are more uncertain because they were inconsistent among *high* and *medium* confidence studies or were evaluated only in *low* confidence studies. Likewise, the available human data were of *low* confidence and difficult to interpret. Integrated evidence of the developmental effects of Cr(VI) exposure from human, animal, and mechanistic studies is summarized in an evidence profile table (Table 3-47).

The evidence of an association between Cr(VI) exposure and developmental effects in humans is *slight*, with an indication of higher rates of spontaneous abortion with higher exposure levels in two of four *low* confidence paternal occupational exposure studies and an ecologic study with exposure at the zip code level (representing both maternal and paternal exposure). Results for other outcomes, including preterm birth, fetal growth, infant death, and congenital malformations indicated no clear association. The available evidence was all considered *low* confidence and the studies generally had poor sensitivity, so there is considerable uncertainty in this judgment.

Animal toxicology studies and supportive mechanistic data provide *moderate* evidence that Cr(VI) exposure leads to developmental effects. The strength of evidence was greatest for effects on

fetal and postnatal growth, which were observed to some extent in the *high* confidence RACB study in mice by NTP (1997) as well as all *low* confidence studies that evaluated these outcomes. The observation of reduced ossification within several *low* confidence studies appears to be consistent with a generalized growth delay, although there is mechanistic evidence suggestive of effects on osteoblasts or thyroid function that could also affect skeletal development. Many studies reported decreased fetal survival and functional effects on the developing reproductive system, but there is more uncertainty in these findings because effects were observed primarily in *low* confidence studies and were not recapitulated in the *high* confidence RACB study by NTP (1997) that evaluated effects through the F2 generation. Other outcomes had limited data available (insulin regulation) or were only evaluated in *low* confidence studies (effects on the placenta) and therefore also have greater uncertainty. Within studies that used a maternal route of exposure, statistically significant effects on fetal development were observed at exposure levels the same or lower than those that caused maternal toxicity. Most studies did not report maternal body weights or other measures of overt toxicity, however, so maternal and fetal toxicity could not be compared within those studies. Decreased fetal survival in paternal-only exposure studies in rats and mice suggests dominant lethal mutations in sperm (as discussed in the "Male reproductive effects" section) and is coherent with human paternal occupational exposure studies. There is more uncertainty in these male-mediated findings because the human and animal studies were rated *low* confidence and effects were not consistent across studies.

Postnatal growth in the RACB study by NTP (1997) was decreased in F1 animals at dose levels of 16.1–37.1 mg/kg-day via diet, and birth weights in F2 females were decreased before adjusting for litter size at 37.1 mg/kg-day Cr(VI). The doses of Cr(VI) at which effects were observed in the *low* confidence drinking water studies in animal models could not be calculated because drinking water consumption data was not reported, and none of the available human studies provided a quantitative measure of exposure. There were no animal studies that evaluated developmental effects following inhalation exposure.

Table 3-47. Evidence Profile Table for Developmental Effects of Cr(VI)

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Evidence from human studies					⊕⊕⊙ The **evidence indicates** that Cr(VI) likely causes developmental effects in humans under relevant exposure circumstances. Decreased offspring growth was observed across most animal studies; other effects were inconsistent in higher confidence studies, had limited data available, or were only evaluated in *low* confidence animal studies. Coherence of spontaneous abortions after paternal occupation exposure in human studies with decreased fetal survival after paternal-only
SPONTANEOUS ABORTION **Low confidence** Hjollund et al. (1995), JP et al. (1992) Hjollund et al. (2000) Hjollund et al. (2005) Remy et al. (2017)	Two studies reported higher rates of spontaneous abortion with higher Cr(VI) exposure and two studies reported lower rates (in one study, the effect varied by analysis).	• Large effect size (RR = 3.5) in one study • Left truncation of early losses could explain inconsistent results	• *Low* confidence studies	⊕⊙⊙ *Slight* Based on associations with paternal occupational exposure and spontaneous abortion in the study with the most sensitive and specific outcome ascertainment (Hjollund et al., 2000).	
OTHER DEVELOPMENTAL EFFECTS **Low confidence** JP et al. (1992) Eizaguirre-García et al. (2000) Peng et al. (2018) Remy et al. (2017)	Two studies reported positive associations between Cr(VI) exposure and preterm birth and birth size. Inconsistent associations reported for congenital malformations.	• No factors noted	• *Low* confidence studies		

Evidence summary and interpretation					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	**Inferences and summary judgment**
Evidence from animal studies					exposure in animal studies; however, only in *low* confidence studies, and effects were not consistent. Mechanistic findings (animals and in vitro) provide supporting evidence of fetal genotoxicity, impaired embryo and fetal functional development, and oxidative stress and apoptosis in the placenta. These mechanisms are presumed relevant to humans.
FETAL AND POSTNATAL SURVIVAL **High confidence:** NTP (1997) Zheng et al. (2018) **Medium confidence:** De Flora et al. (2006) **Low confidence:** Al-Hamood et al. (1998) Arshad et al. (2017) Bataineh et al. (2007) Bataineh et al. (1997) Elbetieha and Al-Hamood (1997) Elsaieed and Nada (2002) Junaid et al. (1995) Junaid et al. (1996b) Kanojia et al. (1998) Marat et al. (2018) Samuel et al. (2012a) Trivedi et al. (1989)	No effects on fetal survival (live pups) in 2 *high* and 1 *medium* confidence studies, including NTP's RACB study in mice. Increased pre- and/or post-implantation loss in 10 *low* confidence studies in which maternal animals were exposed before mating or during gestation. Effects were at doses same or lower than those that caused maternal toxicity. Increased pre- and/or post-implantation loss in 2 out of 4 *low* confidence studies in which only paternal animals were exposed to prior to mating.	• No factors noted	• Effects observed only in *low* confidence studies	⊕⊕⊙ *Moder*ate Based primarily on the observation of decreased offspring growth across most studies, including within the *high* confidence RACB in mice by NTP (1997).	
FETAL AND POSTNATAL GROWTH **High confidence:** NTP (1997) Zheng et al. (2018) **Medium confidence:** De Flora et al. (2006)	Decreased F1 postnatal body weights in NTP's *high* confidence RACB study. Effects on F1 and F2 birth weights in this study were minimal.	• *High* confidence study • Consistency • Effect size • Dose-response gradient	• No factors noted		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Low confidence: Al-Hamood et al. (1998) Arshad et al. (2017) Banu et al. (2017a) Elsaieed and Nada (2002) Junaid et al. (1995) Junaid et al. (1996b) Kanojia et al. (1998) Kumar et al. (2017) Samuel et al. (2012a) Trivedi et al. (1989)	Decreased fetal or pup body weight and other morphometric parameters (e.g., crown-rump length) in 8 out of 9 *low* confidence studies. Within all studies, effects were at same or lower dose levels that those that caused decreased maternal body weight gain.	• Coherence with decreased ossification within *low* confidence studies			
STRUCTURAL ALTERATIONS **Low confidence:** Arshad et al. (2017) Elsaieed and Nada (2002) Junaid et al. (1995) Junaid et al. (1996b) Kanojia et al. (1998) Trivedi et al. (1989) Sánchez et al. (2015)	Decreased fetal skeletal ossification as well as some other structural abnormalities in *low* confidence studies, occurring at the same dose levels as decreased fetal growth. Decreased periodontal bone formation in one *low* confidence study.	• Coherence of decreased ossification with decreased growth	• *Low* confidence studies		
EFFECTS ON THE PLACENTA **Low confidence:** Banu et al. (2017b) Elsaieed and Nada (2002) Junaid et al. (1995)	Histopathological changes in the placenta in 2 *low* confidence studies. Inconsistent effects on placenta weight across studies (increased, decreased or no effect).	• No factors noted	• *Low* confidence studies		

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Junaid et al. (1996b) Kanojia et al. (1998) Trivedi et al. (1989)					
FUNCTIONAL ENDPOINTS **High confidence:** NTP (1997) Zheng et al. (2018) **Medium confidence:** Shobana et al. (2017) **Low confidence:** Al-Hamood et al. (1998) Banu et al. (2008) Banu et al. (2015) Banu et al. (2016) Kumar et al. (2017) Samuel et al. (2012a) Sivakumar et al. (2014) Stanley et al. (2013) Stanley et al. (2014)	Effects on developing male reproductive system observed in 1 *high* confidence study, and effects on developing female reproductive system observed in multiple *low* confidence studies. No effects in NTP's RACB. Increased serum insulin levels and alterations in glucose uptake and glucose oxidation in F1 rats that had been exposed during gestation.	• *High* confidence study • Dose-response gradient • Mechanistic evidence provides biological plausibility	• Unexplained inconsistency across *high* confidence studies		
Mechanistic evidence					
Biological events or pathways	**Summary of key findings and interpretations**			**Judgments and rationale**	
Fetal genotoxicity	*Interpretation:* In vivo evidence of fetal genotoxicity. *Key findings:*			Observations of multiple	

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	• Increased frequency of fetal micronucleated polychromatic erythrocytes when mouse dams were exposed via a single i.p. injection, but no effects following repeat dose oral exposure (De Flora et al., 2006).			mechanisms by which Cr(VI) can disrupt fetal structural and functional development.	
In vitro evaluations of embryo development	*Interpretation:* In vitro evidence that Cr(VI) impairs or arrests embryo development *Key findings:* • Impaired embryo development when Cr(VI)-treated mouse sperm were used to fertilize untreated eggs (Yoisungnern et al., 2015), or when mouse blastocysts were incubated in solutions of Cr(VI) (Iijima et al., 1983; Jacquet and Draye, 1982).				
Mechanisms affecting bone development	*Interpretation:* In vitro evidence that Cr(VI) affects viability and activity of osteoblasts, and in vivo evidence that Cr(VI) decreases thyroid hormone levels. *Key findings:* • Increased cytotoxicity (Ning et al., 2002; Ning and Grant, 2000, 1999) and decreased protein, DNA, RNA, and collagen fiber production (Ning et al., 2002) in an immortalized osteoblast cell line. • Decreased thyroid hormone levels and follicle size in adult male rats exposed via i.p. injection (Qureshi and Mahmood, 2010); this mechanism could affect bone development, but the relevance to developing animals is unclear.				
Mechanisms affecting insulin regulation	*Interpretation:* In vivo evidence that Cr(VI) affects insulin signaling in developing animals. *Key findings:*				

Evidence summary and interpretation					Inferences and summary judgment
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
	• Decreased expression of insulin receptor protein and substrates in F1 offspring from dams exposed via drinking water from GD 9-14 (Shobana et al., 2017).				
Oxidative stress and apoptosis in the placenta	*Interpretation:* In vivo and in vitro evidence that Cr(VI) increases oxidative stress and apoptosis in the placenta. *Key findings:* • Biomarkers of oxidative stress and apoptosis observed in human placenta samples with relatively high Cr levels (Banu et al., 2018), in rat placentas following in vivo oral exposure (Banu et al., 2017b; Banu et al., 2017c), and in placental cells (Banu et al., 2018) or placental mitochondria (Sawicka and Długosz, 2017) cultured in vitro.				

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3.3. SUMMARY OF HAZARD IDENTIFICATION AND CONSIDERATIONS FOR DOSE-RESPONSE ANALYSIS

3.3.1. Susceptible Populations and Life Stages

Susceptible populations and life stages refers to groups of people who may be at increased risk for negative health consequences following chemical exposures due to factors such as life stage, genetics, health status and disease, gender, lifestyle factors, and other co-exposures. This discussion of susceptibility focuses on factors for which there are available Cr(VI) data and factors hypothesized to be important to Cr(VI).

A number of different factors were identified that could predispose some populations of humans to be more susceptible to Cr(VI) toxicity. These factors depend on the toxicity of concern and route of exposure. For all endpoints following oral exposure (GI tract cancer and noncancer, hepatic effects, developmental effects), conditions that elevate stomach pH would lower an individual's ability to reduce Cr(VI) effectively and could lead to a higher rate of Cr(VI) absorption (see Section 3.1). Stomach pH may vary according to health status and life stage. For respiratory effects, preexisting respiratory conditions may be exacerbated by inhalation of Cr(VI). Preexisting GI, liver, and hematologic conditions may be exacerbated by ingestion of Cr(VI).

3.3.1.1. *Health status and disease*

Low stomach acid

Individuals with chronically high stomach pH are expected to detoxify Cr(VI) less effectively, leading to increased uptake of Cr(VI) in the GI tract (affecting the GI and other systemic tissues). Individuals with hypochlorhydria (also known as achlorhydria) have consistently low stomach acid, causing high stomach pH (Kalantzi et al., 2006; Feldman and Barnett, 1991; Christiansen, 1968). This condition may be caused or exacerbated by multiple preexisting gastric conditions, including *H. pylori* infection. Less than 1% of the adult population may exhibit hypochlorhydria, whereas 10–20% of the elderly population (age 65 and up) may exhibit this condition (Russell et al., 1993). In addition, individuals taking medication to treat gastroesophageal reflux disease (GERD), including calcium carbonate-based acid reducers and proton pump inhibitors, have an elevated stomach pH during treatment. Approximately 20% of the population may be afflicted by GERD (Lin and Triadafilopoulos, 2015). Sensitivity analyses on high-pH populations using the PBPK model were performed to inform the dose-response assessment (see Appendix C.1.5).

In addition to those with medical conditions, there is a significant percentage of individuals with high stomach pH due to population variability. Among adults without hypochlorhydria, Feldman and Barnett (1991) estimated that 5% of men may exhibit basal pH exceeding 5, and 5% of women may exhibit basal pH exceeding 6.8. In the healthy elderly population, the percentage of

individuals with pH > 5 may be higher than for adults (Russell et al. (1993) observed that 11% of elderly subjects had pH > 5).

GI tract disease

Individuals with preexisting GI conditions may be at higher risk of Cr(VI)-induced health effects in the GI tract. Cr(VI) contributes to oxidative stress and inflammation in the GI tract. As a result, damage to the gastric and intestinal epithelia due to preexisting inflammatory GI conditions may be exacerbated by oral Cr(VI) exposure. For stomach cancer, preexisting conditions known to increase risk in humans include *H. pylori* bacterial infection (Bessède et al., 2015; Fox and Wang, 2014) and Epstein-Barr virus (CGARN, 2014). Therefore, populations with these preexisting conditions may also represent a population sensitive to Cr(VI)-induced gastrointestinal tract cancer.

Liver diseases

Populations with preexisting liver disease represent a population susceptible to Cr(VI). Cr(VI) contributes to oxidative stress in the liver, causes inflammation, increased fat storage (histologically noted as vacuolation or fatty changes), and substantial increases in serum ALT and AST, indicative of hepatocellular injury (see Section 3.2.4). The most common chronic liver disease in western societies is nonalcoholic fatty liver disease (NAFLD), with an increasing prevalence in line with obesity. It is estimated that 25% of the US population has NAFLD (Younossi, 2019). This condition is characterized by excessive fat accumulation, especially triglycerides, in hepatocytes. If untreated, NAFLD can progress to nonalcoholic steatohepatitis (NASH) and continue to fibrosis, cirrhosis, and in some cases, hepatocellular carcinoma (Monserrat-Mesquida et al., 2020). Increased oxidative stress/pro-inflammatory status is implicated in the pathogenesis of NAFLD (Videla et al., 2004) and increased inflammation is associated with increased severity of NASH (Monserrat-Mesquida et al., 2020). NAFLD is associated with type 2 diabetes, metabolic syndrome, obesity and cardiovascular disease (Younossi, 2019), therefore, populations with these preexisting conditions likely also represent a population sensitive to Cr(VI)-induced liver perturbation.

Respiratory diseases

Inhaled Cr(VI) exposure may exacerbate preexisting respiratory conditions such as asthma, emphysema and chronic obstructive pulmonary disease (COPD). This is because preexisting conditions which reduce lung capacity, inflame airways, or obstruct breathing could be compounded by Cr(VI) exposure, which may induce similar effects. Additionally, respiratory conditions induced by lifestyle factors (i.e., smoking) or co-exposures (i.e., asbestos) may interact with the effects induced by inhaled Cr(VI) exposure.

Anemia and other blood disorders

Because the **evidence suggests** that Cr(VI) may produce anemia-like effects such as reduced hematocrit, hemoglobin, MCV, MCH, and MCHC (see Section 3.2.5), exposure to Cr(VI) may exacerbate the condition in individuals with preexisting conditions such as anemia, iron deficiency or bleeding disorders.

3.3.1.2. *Genetic factors*

Genetic polymorphisms

As summarized in Cancer MOA, Section 3.2.3.3, individuals with genetic polymorphisms conveying deficiencies in DNA repair capacity may have increased susceptibility to Cr(VI)-induced lung cancer. See Section 3.2.3.3 and Appendix C.3.14 for more details (see also Urbano et al. (2012)).

Carriers of a mutated cystic fibrosis transmembrane conductance regulator (CFTR) allele

Suppression of the CFTR gene was shown to enhance intestinal tumorigenesis in animal models (Than et al., 2016). An analysis of the toxicogenomic data reported in Kopec et al. (2012b; 2012a) from mice exposed to Cr(VI) have identified a potential role for CFTR in the carcinogenic effects of Cr(VI) (see Appendix C.3.4.2). Data from Kopec et al. (2012b; 2012a) indicate that CFTR was inactivated in mice exposed to Cr(VI) in drinking water concentrations as low as 0.1 mg/L. In the US, more than 10 million people are carriers of a mutated CFTR allele that confers an approximately 50% reduction in CFTR expression levels; the deficit in CFTR function has been shown to lead to an increased risk for several conditions associated with cystic fibrosis, including colorectal cancer (Miller et al., 2020; Scott et al., 2020). Thus, individuals with this preexisting condition may suffer an even further reduction in CFTR expression levels following oral exposure to Cr(VI).

Heritable adenomatous polyposis coli (APC) mutations cause most cases of familial adenomatous polyposis (FAP), an inherited syndrome associated with a high risk of colorectal cancers (Jasperson et al., 2017; Leoz et al., 2015). Impaired CFTR activity was also shown to enhance intestinal tumorigenesis in mice carrying the mutated tumor-suppressor gene adenomatous polyposis coli (*Apc)*. As a result, carriers of APC mutations may be more susceptible to the tumorigenicity induced by events that inactivate CFTR, including Cr(VI) exposure, and there could be additional risk for individuals carrying both the CFTR and APC mutations. Although 95% of patients with classic FAP develop colorectal cancer by age 35 (Leoz et al., 2015), there are over 1000 different types of APC mutations, many associated with a milder variant of FAP, that would also be affected by CFTR inactivation.

3.3.1.3. *Life Stage*

Developmental stages

Because Cr(VI) was determined to likely cause developmental toxicity in humans given relevant exposure circumstances, pregnant women are considered a sensitive subpopulation. In human studies of Cr(VI) focusing on this population, there are some indications of an association between Cr(VI) exposure and spontaneous abortion, fetal growth, preterm birth, and congenital malformations, but the evidence is limited in quality and quantity (see Section 3.2.9).

Early life stages

Neonates, infants, and young toddlers generally have neutral stomach pH for the first 20–30 months, which then lowers to the normal adult range of 1–2 (Neal-Kluever et al., 2019; Bai et al., 2016). Neonates also have delayed gastric emptying of milk, formula and other caloric-containing liquids (Neal-Kluever et al., 2019). Delayed stomach emptying combined with elevated stomach pH would lead to a higher uptake of ingested Cr(VI) in the stomach. In addition, incomplete stomach reduction would lead to increased uptake of Cr(VI) in the small intestine. For chronic noncancer effects and derivation of the RfD, this short-term change in the potential for absorbed Cr(VI) does not impact the total lifetime average daily absorbed dose (because it occurs during such a short time period). For cancer effects, incorporation of age-dependent adjustment factors in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) account for early-life (<2 years) susceptibility by using a 10-fold adjustment to the slope factor.

Later life stages

In general, healthy elderly men and women (age 65 and older) have similar pH profiles as adults (Russell et al., 1993), although they may have slightly lower stomach pH than adults, and higher duodenal pH (Bai et al., 2016). The healthy elderly population has the same gastric emptying rate as healthy adults, but slower transit time in the small and large intestine (Bai et al., 2016). There are age-related changes in the liver affecting hepatic clearance of drugs (Bai et al., 2016), but it is not clear how this may affect hepatic reduction of Cr(VI). As a result, it is uncertain how Cr(VI) may affect the healthy elderly population differently from adults. However, elderly populations are more likely to have preexisting health conditions that can lead to increased susceptibility to the effects of ingested Cr(VI). The elderly have high prevalence of conditions associated with hyporchlorhydria such as *H. pylori* infection (Bai et al., 2016; Morihara et al., 2001; Russell et al., 1993). The elderly also have higher usage of proton pump inhibitors to treat acid reflux diseases, leading to increased stomach pH (Burdsall et al., 2013). As a result, it is possible that the elderly are more susceptible to the health effects of ingested Cr(VI), but mostly due to pre-existing conditions that are associated with ageing.

3.3.1.4. *Sex*

Males and females can differ greatly in body composition, organ function, and many other physiological parameters that may influence the pharmacokinetics of chemicals and their metabolites in the body (Gochfeld, 2007; Gandhi et al., 2004). On average, males and females are expected to have the same stomach pH (Shih et al., 2003; Dressman et al., 1990). The human epidemiology studies do not support any specific gender susceptibilities for noncancer effects due to Cr(VI) exposure. In animals, GI tract toxicity and hepatotoxicity may have been more severe in females (see Sections 3.2.2 and 3.2.4), but it is unclear if the slight differences in results by gender in rodents are applicable to humans.

3.3.2. Effects Other Than Cancer

Evidence indicates that Cr(VI) is likely to cause GI, liver, developmental, and lower respiratory toxicity in humans, given relevant exposure circumstances. Evidence suggests (but is not sufficient to infer) that Cr(VI) may cause male reproductive, immune, and hematologic toxicity in humans. Evidence is inadequate to assess whether Cr(VI) causes female reproductive toxicity in humans. The evidence base consisted of a wide array of animal and human studies (outlined in greater detail by the health effect summary subsections below). A summary of the justifications for the evidence integration conclusions for each of the main hazard sections is provided below and organized by health effect.

The strength of the evidence for each hazard differed by species and route of exposure. As discussed in Section 3.1, differences in observed effects between routes of exposure can be attributed to pharmacokinetics. There was a lack of sufficient dose-response data for health hazards outside of the respiratory tract following inhalation exposure, and as a result derivation of the RfC was limited to effects in the respiratory tract. Similarly, respiratory tract effects were not observed following oral ingestion, and derivation of the RfD was limited to effects observed following ingestion (GI, hepatic, and developmental effects). Additional considerations, decisions, and rationale are presented below in Table 3-48 and in Sections 4.1 and 4.4.

Table 3-48. Dose response considerations and rationale for specific routes of exposure and health effects

Dose response consideration	Decision	Rationale
Health effects for RfC derivation	RfC derivation for respiratory tract effects only. Route-to-route extrapolation not performed.	Pharmacokinetic differences are significant between inhalation and oral exposure, particularly for portal-of-entry effects.
Animal and human data for RfC derivation	RfC derivation of nasal effects used human data only. RfC derivation of lower respiratory effects used animal data only.	Quantitative dose-response data from medium and high confidence studies were limited by species and effects.

Dose response consideration	Decision	Rationale
Animal and human data for RfD derivation	RfD derivation used animal data only.	Quantitative dose-response data from medium and high confidence oral studies were only available for rodents.
Appropriate exposure data for RfD derivation	Gavage studies excluded. Studies not including a dose group below 20 mg/kg-d excluded.	Concern for frank-effect toxicity.

3.3.2.1. *GI tract effects*

The determination that evidence indicates that Cr(VI) is likely to cause GI toxicity in humans (given relevant exposure circumstances) was based on four high confidence toxicology studies. Two of these studies (NTP, 2008, 2007) contained multiple study arms, resulting in both chronic and subchronic data across multiple species, strains, and sexes (see Table 3-49). All four high confidence studies in rats and mice reported various histological effects in the GI tract associated with oral exposure to Cr(VI). These include diffuse epithelial hyperplasia or crypt cell hyperplasia, histiocytic cellular infiltration, squamous metaplasia, degenerative changes in the villi (vacuolization, atrophy, and apoptosis), and gastric ulceration (Thompson et al., 2012b; Thompson et al., 2011; NTP, 2008, 2007). The literature search for this assessment did not identify epidemiological studies with analyses of GI effects in humans that met PECO criteria.

Mechanistic evidence supports the GI tract effects observed in animals and suggests a possible MOA of Cr(VI)-induced GI toxicity involving the production of free radicals and reactive intermediates through intracellular Cr(VI) reduction resulting in oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis. Degenerative changes to the cells lining the GI tract can manifest as necrosis, apoptosis, and subsequent villous stunting, resulting in crypt abscess and ulceration (Betton, 2013). Irreversible cytoplasmic vacuolization can be a marker of cell death and cytoprotective autophagy in response to stress (Shubin et al., 2016).

The histiocytic cellular infiltration endpoint was not of concern for dose-response analysis because it was also observed in tissues with no indications of degenerative changes or effects. Endpoints observed in subchronic studies such as apoptosis, villous atrophy, and villous cytoplasmic vacuolization are mechanistic and not considered for dose-response assessment.

Diffuse epithelial hyperplasia only occurred in portions of the GI tract where other degenerative effects were observed. Diffuse epithelial hyperplasia, although predictive of more severe manifestations of toxicity, is considered minimally adverse. Data for this endpoint are available from both the chronic and subchronic studies (Table 3-49).

Table 3-49. Available animal studies showing histopathological changes in the duodenum

Reference	Study arms performed	Observations
NTP (2008)	F344 Rat, male and female (chronic)	Histiocytic cellular infiltration.
	B6C3F1 mouse, male and female (chronic)	Diffuse epithelial hyperplasia.
		Histiocytic cellular infiltration.
NTP (2007)	F344 Rat, male and female (subchronic)	Histiocytic cellular infiltration.
	B6C3F1 mouse, male and female (subchronic)	Epithelial hyperplasia, histiocytic cellular infiltration.
	B6C3F1, BALB/c, and am-C57BL/6 mouse, male (subchronic strain comparison)	Epithelial hyperplasia, histiocytic cellular infiltration.
Thompson et al. (2012b)	F344 Rat, female (subchronic)	Crypt cell hyperplasia, histiocytic infiltration, apoptosis, villus atrophy
Thompson et al. (2011)	B6C3F1 mouse, female (subchronic)	Crypt cell hyperplasia, histiocytic infiltration, apoptosis, villus atrophy, villous cytoplasmic vacuolization

3.3.2.2. *Hepatic effects*

The determination that evidence indicates that Cr(VI) is likely to cause hepatic toxicity in humans (given relevant exposure circumstances) was based on studies in animals that observed hepatic effects following drinking water exposure. Several studies in rats and mice reported various histological lesions in the liver associated with oral exposure to Cr(VI). These lesions include increased inflammation and infiltration of immune cells, fatty changes and vacuolation, indications of apoptosis and necrosis, and increased incidence of altered hepatic foci. NTP (2008) described chronic inflammation as "minimal to mild severity" in most dose groups, with "mild to moderate" in the higher dose groups. The severity ratings were used to inform BMR selection (see Section 4.1).

Many studies have examined serum indicators that are potentially informative for predicting hepatotoxicity following exposure to Cr(VI). The most commonly reported indicators included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and sorbitol dehydrogenase (SDH). These changes were observed across multiple studies, with ALT changes exceeding twofold which is an indicator of concern for hepatic injury (Sawicka and Długosz, 2017; EMEA, 2010; Boone et al., 2005). The outcomes rated *medium* confidence showing a response were available from chronic and subchronic studies across multiple species, strains, and sexes (see Table 3-50). These are discussed further in Section 4.1.

The human evidence for Cr(VI)-induced liver effects is limited in terms of number and confidence of studies. However, two of the available three studies (one occupational and one general population study) provide some indication of exposure-related alterations of liver clinical chemistry (Sazakli et al., 2014; Saraswathy and Usharani, 2007).

Mechanistic evidence supports the hepatic effects observed in animals and humans and suggests a possible MOA of Cr(VI)-induced liver toxicity involving the production of free radicals and reactive intermediates through intracellular Cr(VI) reduction resulting in oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis.

Table 3-50. Available animal studies showing histopathological and clinical chemistry changes in the liver

Reference	Species/strain and sex	Observations*
NTP (2008)	F344 Rat, male and female (chronic)	Histopathology: histiocytic cellular infiltration, chronic inflammation, fatty change, basophilic focus Clinical chemistry (male rats only): ALT, ALP, SDH, bile acids
	B6C3F1 mouse, male and female (chronic)	Histopathology: histiocytic cellular infiltration, chronic inflammation
NTP (2007)	F344 Rat, male and female (subchronic)	Histopathology: histiocytic cellular infiltration, chronic focal inflammation
		Clinical chemistry: ALT, ALP, SDH, bile acids, cholesterol, triglycerides, 5'nucleotidase
	B6C3F1, BALB/c, and am-C57BL/6 mouse, male (subchronic)	Clinical chemistry: ALT, ALP, SDH, bile acids, glycogen (B6C3F1 and am-C57BL/6 only)
Rafael et al. (2007)	Wistar rat, male (chronic)	Clinical chemistry: ALT, ALP, SDH, glucose, cholesterol, total protein
NTP (1996a)	BALB/c mouse, male and female (subchronic)	Histopathology: cytoplasmic vacuolation (fatty change)
NTP (1997)	BALB/c mouse, male and female (continuous breeding)	Histopathology: hepatocyte cytoplasmic vacuolation (fatty change), hepatocyte individual cell necrosis, necrosis, acute inflammation
Krim et al. (2013)	Wistar rat, male (subchronic)	Clinical chemistry: ALT, ALP, AST, cholesterol, total lipids, triglycerides, LDH
Wang et al. (2015)	Sprague-Dawley rat, male (subchronic)	Clinical chemistry: ALT, AST, cholesterol, triglycerides, glucose
Navya et al. (2017a)	Wistar rat, male (subchronic)	Clinical chemistry: ALT, ALP, AST

*Only endpoints rated medium or high confidence within each study are listed

3.3.2.3. *Respiratory tract effects*

The determination that evidence indicates that Cr(VI) is likely to cause respiratory toxicity in humans (given relevant exposure circumstances) was based on studies in animals that observed effects following inhalation exposure. Most animal inhalation studies of lower respiratory effects contained data for lung histopathology, lung weight, and cellular responses. Because histopathological and cellular changes occurred together, and in combination with serum biomarkers indicating an inflammatory response (Nikula et al., 2014), these were considered indicators of adverse responses and considered for dose-response analysis. Because lung weight is

a nonspecific endpoint for lung injury (e.g., lung weight increase in the only medium confidence data by Glaser et al. (1985) may be related to accumulation of macrophages), this endpoint was not considered for dose-response analysis. The available histopathological changes and cellular response outcomes that were rated medium confidence are outlined in Table 3-51. These are discussed further in Section 4.2.

The human evidence for Cr(VI)-induced lower respiratory effects is limited in terms of number and confidence of studies. However, two of the available four studies provide some indication of exposure-related decrements in lung function assessed using spirometry (Li et al., 2015b; Kuo et al., 1997b).

Mechanistic evidence supports the respiratory tract effects observed in animals and suggests a possible MOA of Cr(VI)-induced toxicity involving the production of free radicals and reactive intermediates through intracellular Cr(VI) reduction resulting in oxidative stress.

Table 3-51. Available animal studies showing histopathological changes and cellular responses in the lung

Reference	Species/ strain and sex	Observations*
Glaser et al. (1990)	Wistar rat, male (subchronic)	Histopathology: Histiocytosis, bronchioalveolar hyperplasia, fibrosis
		BALF: LDH, ALB, total protein, macrophage effects
Glaser et al. (1985)	Wistar rat, male (subchronic)	BALF: Macrophage effects
Johansson et al. (1986a)	Rabbit, male (subchronic)	Histopathology: Histiocytosis
Cohen et al. (2003)	F344 Rat, male (chronic)	BALF: Total cells, total macrophages
Johansson et al. (1986b)	Rabbit, male (subchronic)	BALF: Total macrohpages, macrophage effects
Kim et al. (2004)	Sprague-Dawley Rat, male (subchronic)	Histopathology: Inflammatory markers (qualitative)

*Only endpoints rated medium or high confidence within each study are listed

3.3.2.4. *Developmental effects*

The determination that evidence indicates that Cr(VI) is likely to cause developmental toxicity in humans (given relevant exposure circumstances) was based on the observation of decreased offspring growth across most animal studies, as evidenced by decreased fetal or postnatal body weights and decreased skeletal ossification. The only data suitable for dose-response analysis were for fetal and postnatal growth, which were observed to some extent in the high confidence RACB study in mice by NTP (1997) (all other studies were low confidence and not considered for dose-response assessment). Within the animal studies, statistically significant effects on fetal development were observed at doses the same or lower than those that caused decreased maternal body weight. According to EPA Guidelines, developmental effects at doses that

cause minimal maternal toxicity are still considered to represent developmental toxicity and should not be discounted as maternal toxicity (U.S. EPA, 1991). Because of the correlation between maternal dam weight and offspring body weight, the maternal dose was used as the basis for dose-response modeling instead of the averaged F0 male and female dose.

3.3.3. Cancer

Under the 2005 Guidelines for Carcinogen Risk Assessment, Cr(VI) is "carcinogenic to humans". This determination applies to both inhalation and oral routes of exposure.

In 1998, the EPA IRIS Toxicological Review of Hexavalent Chromium classified Cr(VI) as a "known human carcinogen by the inhalation route of exposure" based on consistent evidence that inhaled Cr(VI) causes lung cancer in humans and supporting evidence of carcinogenicity in animals. The same conclusion has since been reached by other authoritative federal and state health agencies and international organizations and the carcinogenicity of Cr(VI) is considered to be well-established for inhalation exposures (TCEQ, 2014; IPCS, 2013; NIOSH, 2013b; IARC, 2012; CalEPA, 2011; NTP, 2011; OSHA, 2006). As stated in the 2014 preliminary packages (U.S. EPA, 2014b, c) and the Systematic Review Protocol (Appendix A), the review of cancer by the inhalation route focused on data that may improve the quantitative exposure-response analysis conducted in EPA's 1998 IRIS assessment. An overview of the literature screening for exposure-response data is contained in Section 4.4.

Determination that Cr(VI) is carcinogenic to humans by the oral route of exposure was made based on 1) a high confidence study in rodents showing a clear dose-response relationship between oral Cr(VI) exposure and incidence of GI tract tumors (NTP, 2008); 2) a meta-analysis of Cr(VI) exposure[55] in relation to GI tract cancers which found a statistically significant increase in risk for colon and rectal cancer in humans (see Section 3.2.3); and 3) robust evidence that a mutagenic MOA has a key role in Cr(VI)-induced cancer via inhalation and oral exposures (see Section 3.2.3).

Because a mutagenic mode of action for Cr(VI) carcinogenicity (see Section 3.2.3) is "sufficiently supported in (laboratory) animals" and "relevant to humans," EPA uses a linear low dose extrapolation from the POD in accordance with Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a). Furthermore, in the absence of chemical-specific data to evaluate differences in age-specific susceptibility, increased early-life susceptibility to hexavalent chromium is assumed and EPA applies ADAFs in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). Linear low dose extrapolation and

[55]The exposure route for human studies in the meta-analysis was primarily inhalation. However, the findings from the meta-analysis for cancer of the GI tract were used to support the mode of action analysis and human relevance for the tumor site.

application of ADAFs are considered for both the inhalation and oral routes of exposure[56]. An overview of the datasets available for dose-response modeling of tumors in the GI tract is provided below in Table 3-52.

Table 3-52. Available animal studies showing increased tumor incidence following ingestion

Reference	Study arms performed	Observations
NTP (2008)	F344 Rat, male and female (chronic)	Dose-dependent increase in tumors of the oral cavity.
	B6C3F1 mouse, male and female (chronic)	Dose-dependent increase in tumors of the small intestine.

Due to reduction (detoxification) of Cr(VI) in the stomach compartment prior to transit to the small intestine, dose-response modeling of tumors in the mouse small intestine incorporates adjustments by a PBPK model when performing animal-to-human extrapolation. For tumors of the rat oral cavity, PBPK modeling is not applied, because Cr(VI) in drinking water exposes the epithelium of the tongue and oral mucosa prior to detoxification in the stomach.

[56]Because carcinogenicity determination was not performed for lung cancer, this section focuses only on cancer of the GI tract. A discussion of the considerations for dose-response of lung cancer is contained in Section 4.4.

4. DOSE-RESPONSE ANALYSIS

4.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from points of departure (PODs) such as a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

As discussed in Sections 3.2.2, 3.2.4, 3.2.5, and 3.2.9, based on findings in experimental animals, the **evidence indicates** that exposure to Cr(VI) is likely[57] to cause GI, liver, and developmental toxicity in humans. Organ-specific reference values were derived for these effects.

4.1.1. Identification of Studies for Dose-Response Analysis of Selected Effects

In order to identify the studies for dose-response analysis, key attributes of the studies reporting the endpoints selected for each hazard were reviewed (i.e., study size and design, relevance of the exposure paradigm, and measurement of the endpoints of interest). Exposure paradigms including a relevant route of human environmental exposure are preferred. When developing a chronic reference value, chronic or subchronic studies are preferred over studies of acute exposure durations (with the exception of developmental studies, where exposures only need to occur during susceptible periods). Studies with a broad exposure range and multiple exposure levels are preferred to the extent that they can provide information about the shape of the exposure-response relationship.

Human studies are generally preferred over animal studies as the basis for a reference value when quantitative measures of exposure are reported, and the reported effects are determined to be associated with exposure. The available epidemiological studies of worker populations exposed to Cr(VI) examined the relationship between certain health endpoints and inhalation exposure; however, no sufficient epidemiological studies of ingested Cr(VI) are available and route-to-route extrapolation was not considered for this assessment (see Protocol, Appendix A). In the absence of human data, the animal studies were considered for dose-response analysis.

Experimental animal studies considered for each health effect were evaluated using general study quality considerations discussed in the Protocol (Appendix A). The oral animal toxicological

[57]Reference values were generally not derived for hazards where only evidence suggests (but is not sufficient to infer) that Cr(VI) may induce health effects.

evidence base for Cr(VI) consists of chronic and subchronic studies. Because *medium* and *high* confidence studies were available, *low* confidence studies were not considered for toxicity value derivation.

Cr(VI) can induce frank effects in rodents at high doses, which raises considerations of exposures and study designs appropriate for dose-response analysis. Because Cr(VI) gavage exposure has been shown to induce frank effects and high mortality in rodents (gut detoxification is much less effective for gavage exposure), these studies were not considered for dose-response assessment. This criterion resulted in the omission of one *high* confidence study (Zheng et al., 2018) from consideration of dose-response analysis for developmental effects. High dose exclusion criteria for drinking water and oral feed studies were also considered. At approximately 20 mg/kg-d *ad libitum*, NTP (2007) reported reduced body weight, chemical-induced stomach ulcers (80–100% incidence), and reduced water consumption in rats exposed for 90 days. The study also reported 10–20% decreases in final body weight relative to controls in mice exposed for 90 days at the high doses (approximately 15–25 mg/kg-d). In order to focus on chronic effects observed in the low dose region (defined here as around 1 mg/kg-d *ad libitum* based on results observed by the chronic 2-year NTP (2008) drinking water bioassay), studies which did not include an exposed group below 20 mg/kg-d were not considered for candidate RfD derivation. This criterion ultimately did not impact any decisions regarding dose-response, because all such studies were rated *low* confidence.

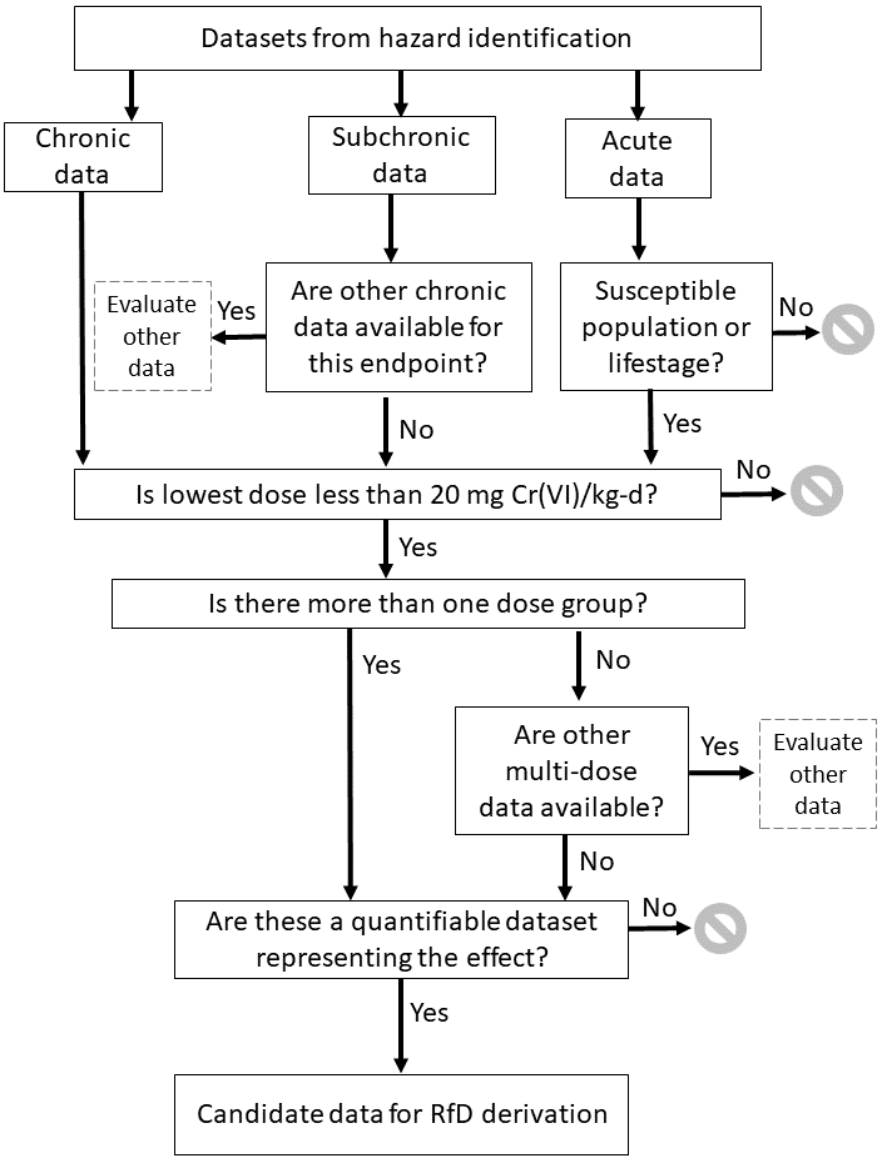

Figure 4-1. Evaluation of studies from the Cr(VI) hazard identification for derivation of toxicity values. For endpoints where *medium* or *high* confidence studies were available, *low* confidence studies were not considered.

4.1.1.1. *GI tract toxicity*

Small intestine histopathology was considered for dose-response analysis of the GI tract effects of oral exposure to Cr(VI). Chronic data from the NTP (2008) 2-year bioassay were used for the dose-response assessment. The chronic 2-year NTP (2008) bioassay analyzed many of the same endpoints as other shorter term studies (which had smaller sample sizes and typically used higher doses). Thompson et al. (2012b; 2011) were subchronic studies which incorporated lower doses than NTP (2008). However, these studies used smaller sample sizes and shorter exposure durations

than NTP (2008), and only examined females (Table 4-1). An overview of design features of the *medium* and *high* confidence animal studies containing data for the GI tract is provided below in Table 4-1.

Table 4-1. Design features of studies that examined GI tract effects (histopathology) via the oral route of exposure

Study reference	Species/strain and sex	Exposure duration	Number of dose groups[b]	Number of animals/group	Dose range (mg/kg-d)
NTP (2008)[a]	B6C3F1 mouse, male and female	2 years	4	50	0.3–8.9
NTP (2008)	F344 Rat, male and female	2 years	4	50	0.2–7.1
NTP (2007)	F344 Rat, male and female	90 days	5	10	1.7–21
NTP (2007)	B6C3F1 mouse, male and female	90 days	5	10	3.1–27.9
NTP (2007)	B6C3F1 mouse, male	90 days	3	5	2.8–8.7
NTP (2007)	BALB/c mouse, male	90 days	3	5	2.8–8.7
NTP (2007)	am-C57BL/6 mouse, male	90 days	3	5	2.8–8.7
Thompson et al. (2012b)	F344 Rat, female	90 days[c]	5	10	0.015–20
Thompson et al. (2011)	B6C3F1 mouse, female	90 days[c]	6	10	0.024–31.1

[a]Preferred data for dose-response.
[b]Number does not include control group.
[c]Note: Thompson et al. (2012b) and Thompson et al. (2011) also performed an 8-day sacrifice on 5 animals/group.

The most sensitive GI effect in mice, diffuse epithelial hyperplasia, was consistently observed at statistically significant incidence levels in mice in all exposure groups (≥0.3 mg/kg-d Cr(VI)) of males and females of multiple strains in three *high* confidence subchronic and chronic studies (Thompson et al., 2011; NTP, 2008, 2007). The hyperplastic duodenal lesions were described as being suggestive of tissue regeneration following degenerative changes to the intestinal villi. In rats, it was observed less consistently and at higher doses compared to mice (Cullen et al., 2015; Thompson et al., 2012b; Thompson et al., 2011). Dose-response modeling was performed on the chronic 2-year data for male and female mice exhibiting diffuse epithelial hyperplasia of the proximal small intestine (duodenum).

4.1.1.2. *Hepatic toxicity*

Liver histopathology changes and serum biomarkers of liver injury were considered for dose-response analysis of the hepatic effects of oral exposure to Cr(VI). These were considered the most representative indicators of hepatic toxicity in the database. Fatty liver changes (cytoplasmic

vacuolation) and increased ALT are also clinical markers used in diagnosis of human liver diseases (see Section 3.3). Dose-response modeling was not performed on liver weight because only moderate changes were observed (see Section 3.2.4), and changes in liver histopathology and serum biomarkers were more consistently observed and more sensitive than liver weight changes.

Generally consistent elevations of ALT (biomarkers of liver injury) were seen across various multiple well-conducted studies in both rats and mice, with the magnitude of change considered to be biologically significant and a specific indication of liver damage. For dose-response modeling of clinical chemistry changes, NTP (2008) observed increased alanine aminotransferase (ALT) in male F344 rats at all three data collection time points (3, 6, and 12 months). Dose-response modeling was performed on the clinical chemistry endpoint ALT in male F344 rats[58] at the 12-month and 90-day collection periods of the NTP (2008) bioassay. ALT changes in male and female rats from the 90-day NTP (2007) study were also modeled[59]. ALT changes in male rats at the 90-day timepoint from the 2-year NTP (2008) study were modeled to provide a comparison with the 90-day NTP (2007) data. In mice, changes in ALT only occurred at high doses during the 90-day NTP (2007), and there were no changes in the other clinical chemistry parameters like there were in rats. Therefore, this endpoint was not modeled in mice.

For histopathological changes, increased incidence of chronic liver inflammation was observed in rodents during the 2-year NTP (2008) bioassay, but this endpoint exhibited a monotonic dose-response relationship for female rats and mice. In male rats, the increased inflammation was nonmonotonic and only significantly increased for one dose group. In male mice, no effect was observed. Fatty liver changes were also observed in female rats during the 2-year NTP (2008) bioassay. Similar to the chronic inflammation endpoint, this effect was not consistently observed across species or sex. Dose-response modeling was performed on the incidence data for chronic liver inflammation and fatty liver changes in female rats from NTP (2008), and chronic inflammation in female mice from NTP (2008).

An overview of design features of the *medium* and *high* confidence animal studies containing data for hepatic effects considered for oral dose-response is provided below in Table 4-2. Because there were studies that were rated *high* and *medium* for endpoints within this domain (see Section 3.2.4), *low* confidence studies were not considered for dose-response assessment.

[58]The NTP (2008) 2-year study did not obtain clinical chemistry data in mice or female rats, whereas the 90-day NTP (2007) study contained data for both male and female F344 rats and mice.

[59]Note: the lowest dose (in mg/kg-d Cr(VI) was the same in males and females for the subchronic study. When taking into consideration differences in body weight in the pharmacokinetic model, the daily absorbed dose in males was slightly higher than females (see Appendix C.1.5).

Table 4-2. Design features of studies that examined hepatic effects (clinical chemistry and histopathology) via the oral route of exposure

Study reference (quality)	Species/strain and sex	Exposure duration	Number of dose groups[b]	Number of animals/group	Dose range (mg/kg-d)
NTP (2008) (high)[a]	F344 Rat, male and female	2 years	4	50	0.2–7.1
NTP (2008) (high)[a]	B6C3F1 mouse, male and female	2 years	4	50	0.3–8.9
NTP (2007) (high)[a]	F344 Rat, male and female	90 days	5	10	1.7–21
NTP (2007) (high)	B6C3F1 mouse, male and female	90 days	5	10	3.1–27.9
NTP (2007) (high)	B6C3F1 mouse, male	90 days	3	5	2.8–8.7
NTP (2007) (high)	BALB/c mouse, male	90 days	3	5	2.8–8.7
NTP (2007) (high)	am-C57BL/6 mouse, male	90 days	3	5	2.8–8.7
Navya et al. (2017a) (medium)	Wistar rat, male	28 days	1	6	10.6
Rafael et al. (2007) (medium)	Wistar rat, male	10 weeks	1	9 control, 19 exposed	2.96
NTP (1996a) (high)	BALB/c mouse, male and female	9 weeks	4	24 males, 48 females (5–6 males, 12 females/group per timepoint)	1.1–48.4
NTP (1997) (high)	BALB/c mouse, male and female	13-week continuous breeding	3	20 (F0), 5–10 (offspring)	6.8–50
Krim et al. (2013) (medium)	Wistar rat, male	30	1	10	5.3
NTP (1996b) (high)	Sprague-Dawley rat, male and female	9 weeks	4	5	0.35–9.90
Wang et al. (2015) (medium)	Sprague-Dawley rat, male	4 weeks	3	8	2.5–7.6

[a]Preferred data for dose-response.
[b]Number does not include control group.

In summary, dose-response modeling was performed on the following hepatic datasets:

4) Increased ALT in male rats from NTP (2008) at the 90-day timepoint and 12-month timepoint

5) Increased ALT in male and female rats from NTP (2007) (90 days)[60]
6) Increased chronic liver inflammation in female rats from NTP (2008) (2 years)
7) Increased chronic liver inflammation in female mice from NTP (2008) (2 years)
8) Fatty liver change in female rats from NTP (2008) (2 years)

4.1.1.3. *Developmental toxicity*

As noted in Section 3.2.9, decreases in fetal and postnatal growth were observed in exposed animals, and these were the only consistently-observed effects. The only two *medium* to *high* confidence studies that observed this effect was NTP (1997). De Flora et al. (2006) did not observe this effect. The high confidence study by Zheng et al. (2018) was not considered for dose-response assessment because it was a gavage study (see 4.1.1). Dose-response modeling was performed on fetal and postnatal growth outcomes in the F1 generation observed by NTP (1997). Data are available for males (PND14 and PND21) and females (PND14 and PND21).

4.1.2. Methods of Analysis

Biologically based dose-response models are not available for Cr(VI). In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, EPA evaluated dose-response information with the models available in EPA's Benchmark Dose Software (BMDS, Version 3.2). EPA estimated the benchmark dose (BMD) and the 95% lower confidence limit on the BMD (BMDL) using a benchmark response (BMR) that represents a minimal, biologically significant level of change (U.S. EPA, 2012b). Endpoint-specific BMRs are described below. Where modeling was feasible, the estimated BMDLs were used as points of departure (PODs); the PODs are summarized in Table 4-3. Further details including the modeling output and graphical results for the model selected for each endpoint can be found in Appendix D.1. Where dose-response modeling was not feasible, no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) were identified; NOAELs and LOAELs are also summarized in Table 4-3.

4.1.2.1. *PBPK modeling and animal-to-human extrapolation*

Following ingestion, extracellular reduction of Cr(VI) to Cr(III) in the stomach is a major pathway for detoxification in both rodents and humans, and may have a significant impact on the amount of Cr(VI) available for absorption and distribution. Uptake of Cr(VI) into tissues and intracellular reduction occurs rapidly (see Section 3.1.1 and Appendix C.1.1 for overview). While GI tract PBPK models are capable of estimating the extent of extracellular reduction in the stomach, the in *vivo*

[60]While chronic data are preferred for dose-response, only chronic male data were available for this endpoint. Subchronic data from both the 90-day study and 2-year study were modeled to evaluate possible difference between sexes.

estimates of localized uptake and reduction of Cr(VI) in GI and systemic tissues exhibit high uncertainties (particularly for the distal GI). Thus, all unreduced Cr(VI) that escapes stomach reduction and enters the small intestine (estimated by PBPK modeling) is assumed to have the potential for absorption into epithelial cells. The unreduced mg/kg-d Cr(VI) dose escaping stomach reduction in the rodent can be adjusted to an internal dose[61] by allometric scaling consistent with *Recommended Use of Body Weight$^{3/4}$ as the Default method in derivation of the oral reference dose* (U.S. EPA, 2011c). This assumes that absorbed Cr(VI) is rapidly cleared (reduced or excreted), with interspecies differences following allometry. While there is some uncertainty in how much of the unreduced Cr(VI) escaping the stomach is reduced and absorbed by the GI tissue prior to systemic distribution, the interspecies difference in this amount is likely to be low in relation to the interspecies difference in gastric reduction (which is driven by differences in stomach pH and Cr(VI) reduction capacity).

PBPK modeling revealed that the Cr(VI) dose escaping stomach reduction (and therefore the internal dose) increased linearly with oral dose for rats and mice (Appendix C.1.5). Therefore, performing BMD modeling on the orally administered doses and performing PK conversions at a later step would ultimately produce the same POD as if BMD modeling was performed on the basis of internal PK-derived rodent doses. For humans, gastric reduction is nonlinear with respect to ingested dose (Appendix C.1.5).

The steps for candidate RfD derivation are outlined below and in Figure 4-2:

1) Dose-response modeling was performed on the basis of mg/kg-d Cr(VI) ingested to determine a BMDL or LOAEL/NOAEL. Where possible, time-weighted average daily doses calculated from time-course data (through the time of data collection) were used. For example, for endpoints only measured at the 12-month time point in a 2-year study, the time-weighted average daily doses over 12 months were used for dose-response (as opposed to the average daily doses over the full 2-year study).

2) The BMDL or LOAEL/NOAEL (in units of mg/kg-d Cr(VI)) was converted to an internal dose using the PK model. The internal dose was the average rodent dose escaping reduction (in mg/kg-d) multiplied by $(BW_A/BW_H)^{1/4}$ in accordance with *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c). Study-specific time-weighted average body weights relevant to the data collection time were used in the model and for the BW scaling step.

3) The human PBPK model was used to estimate the daily mg/kg Cr(VI) dose that must be ingested to achieve the internal dose calculated in step (2). To account for interindividual variability, the human equivalent dose was determined by Monte Carlo analysis. The lower

[61]Alternatively for the small intestine, an internal dose to the small intestine may be derived by scaling the un-reduced daily Cr(VI) intake rate by intestinal tissue volume (defined as pyloric flux, mg/L-d, by Thompson et al. (2014)). Because organ volumes vary between species by allometric relationships, using the pyloric flux internal dose metric produces similar results as $BW^{3/4}$ scaling of the un-reduced Cr(VI) dose.

1% value of 20000 Monte Carlo PK simulations needed to achieve the internal dose POD was used. As a result, the intraspecies uncertainty factor (UF_H) was lowered from 10 to 3 (the pharmacokinetic component of the uncertainty factor was removed as it was accounted for with this analysis). See Appendix C.1.5.

4) The uncertainty factors are applied to derive the candidate RfD.

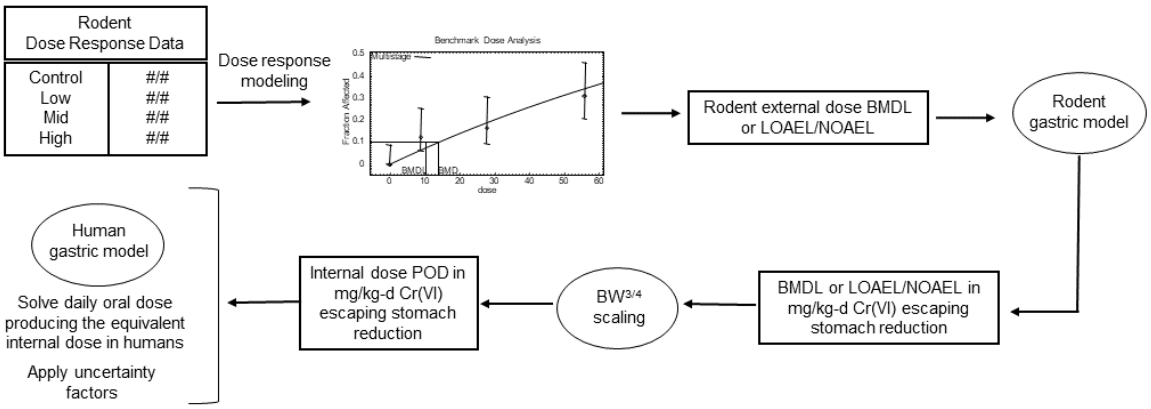

Figure 4-2. Process for calculating the human equivalent dose for Cr(VI).

4.1.2.2. *GI tract Effects*

Incidence data of diffuse epithelial hyperplasia of the duodenum in male mice from NTP (2008) were amenable to BMD modeling with the highest dose omitted. A BMR of 10% extra risk (ER) was applied under the assumption that it represents a minimally biologically significant level of change in the absence of a biologically based BMR (U.S. EPA, 2012b). Diffuse epithelial hyperplasia, although predictive of more severe manifestations of toxicity, is considered minimally adverse and does not support using a lower BMR. Incidence data for male mice (all doses included) are contained in HAWC.

Diffuse epithelial hyperplasia was not amenable to BMD modeling for female mice because there was too much uncertainty in estimating the BMDL (see Appendix D.1.1). There were three models which adequately fit the data in accordance with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). However, they produced significantly different BMDs and BMDLs, and one model did not produce useful results due to an extremely low BMDL estimate and high BMD:BMDL ratio. This is an indication that there was some model dependence of the estimates, and uncertainty in the estimates was too great to be able to rely on the modeling results. The uncertainty was primarily caused by the fact that the observed percent incidence at the lowest dose (38%) was much higher than the BMR (10%). Alternative modeling approaches were explored, however they could not address the lack of low dose data near the target 10% extra risk response level. As a

result, the LOAEL approach was used (the LOAEL for hyperplasia in female mice was 0.302 mg/kg-d). Click here to see incidence data in HAWC for female mice.

4.1.2.3. *Hepatic Effects*

For the liver, data for chronic liver inflammation in female mice from NTP (2008) were amenable to BMD modeling. A BMR of 10% extra risk (ER) was applied under the assumption that it represents a minimally biologically significant level of change. NTP (2008) described these lesions as "minimal to mild severity", with "mild to moderate" in the higher dose groups. As a result, a BMR lower than 10% was not considered.

Changes in the liver enzyme alanine aminotransferase (ALT) at 12 months in male rats from NTP (2008) were amenable to BMD modeling. Several expert organizations, particularly those concerned with early signs of drug-induced hepatotoxicity, have identified an increase in liver enzymes compared with concurrent controls of two to fivefold as an indicator of concern for hepatic injury (Sawicka and Długosz, 2017; EMEA, 2010; Boone et al., 2005; Group, 2000). For this assessment, a twofold increase in ALT is considered indicative of liver injury in experimental animals. Thus, a BMR of 100% change from control (1 relative deviation from control) was applied. Data for male and female rats in the subchronic study by NTP (2007) were not amenable to BMD modeling[62], and the lowest dose was identified as the LOAEL. The chronic study by NTP (2008) also provide subchronic data for ALT in male rats at 90 days. Because the chronic study used lower doses, it was possible to identify a NOAEL[63] of 1.46 mg/kg-d, and a LOAEL of 4.30 mg/kg-d for increased ALT in male rats at 90 days (see Appendix C for time-weighted average daily doses of the first 90 days of exposure during the NTP (2008) 2-year study).

Fatty liver change in female rats from NTP (2008) was not amenable to BMD modeling. Similar to hyperplasia in the female mouse duodenum, uncertainty in estimating the BMDL was too high (see Appendix D.1.1). As a result, the NOAEL (the lowest dose level, 0.248 mg/kg-d, which exhibited less than 10% extra risk) was used as the POD for this dataset. Similarly, chronic liver inflammation in female rats from NTP (2008) was not amenable to BMD modeling and the LOAEL (0.248 mg/kg-d, which exhibited greater than 10% extra risk) was used as the POD.

4.1.2.4. *Developmental Effects*

For NTP (1997), doses reported for the F0 dams[64] were 11.6, 24.4, and 50.6 mg/kg-d Cr(VI) (via feed). Decreased postnatal growth in the F1 generation was observed beginning at 24.4 mg/kg-d. Data are available for males (PND14 and PND21) and females (PND14 and PND21). For postnatal

[62]For female rats, the first nonzero dose had a very high response relative to other dose levels (click here to see dose-response data). For male rats, the goodness-of-fit p-values were less than 0.1 for all statistical models (even when removing the highest dose, which had a low response relative to other exposure levels). Click here to see dose-response data for male rats.

[63]Data were not amenable to BMD modeling. No change from control was observed at the first nonzero dose.

[64]Maternal dam weight is highly correlated to offspring body weight. Because maternal body weight in this study was also decreased, maternal dose is examined here instead of the averaged F0 male and female dose.

growth in the F2 generation, effects were observed at the highest dose only (maternal doses for females in the F1 generation were 7.27, 17.19, 39.15 mg Cr(VI)/kg-d). Datasets for postnatal growth were not amenable to BMD modeling because study statistics reported by the authors were inadequate for use in multi-generational modeling[65]. A NOAEL of 11.6 mg/kg-d was used based on outcomes observed in the F1 generation (see Section 3.2.9).

4.1.3. Derivation of Candidate Values

This section describes the data and rationale for the selection of uncertainty factors and derivation of candidate values for each identified human health hazard. The dose-response modeling results and rodent-to-human extrapolations are summarized in Table 4-3. Further details, including the BMDS modeling output and graphical results for the model selected for each endpoint, can be found in Appendix D.1.

Table 4-3. Summary of derivation of points of departure following oral exposure

Species/ sex	Model	BMR	BMD mg/kg-d	BMDL mg/kg-d	Internal dose[a] mg/kg-d	TWA BW kg	$BW^{3/4}$ adjust[b]	POD_{HED} mg/kg-day[c]
Diffuse epithelial hyperplasia of the duodenum at two years (NTP, 2008)								
Mice/M	Quantal linear[d]	10% ER	0.148	0.121	0.0182	0.05	2.88×10^{-3}	0.0443
Mice/F	LOAEL	--	--	0.302	0.0463	0.05	7.32×10^{-3}	0.0911
Changes in the liver enzyme alanine aminotransferase (ALT) (NTP, 2008)								
Rat/M 12 mo	Expon.2 [d]	1RD	1.82	1.55	0.168	0.395	0.0445	0.206
Rat/M 3 mo	NOAEL	--	--	1.46	0.149	0.246	0.0351	0.184
Changes in the liver enzyme alanine aminotransferase (ALT) at 90 days (NTP, 2007)								
Rat/M	LOAEL	--	--	1.74	0.188	0.232	0.0436	0.203
Rat/F	LOAEL	--	--	1.74	0.181	0.160	0.0383	0.190
Chronic liver inflammation at two years (NTP, 2008)								
Rat/F	LOAEL	--	--	0.248	0.0195	0.260	4.66×10^{-3}	0.0669
Mice/F	Log-logistic	10% ER	3.70	1.33	0.225	0.05	0.0356	0.182
Liver fatty change at two years (NTP, 2008)								
Rat/F	NOAEL	--	--	0.248	0.0195	0.260	4.66×10^{-3}	0.0669
Decreased offspring growth (NTP, 1997)								

[65]It was unclear whether standard errors reported for dose groups are based on variation among litters or among pups across litters, and individual-level data are not available.

Species/ sex	Model	BMR	BMD mg/kg-d	BMDL mg/kg-d	Internal dose[a] mg/kg-d	TWA BW kg	$BW^{3/4}$ adjust[b]	POD_{HED} mg/kg-day[c]
Mouse/F	NOAEL	--	--	11.6	3.09	0.0240	0.407	0.700

[a]Dose escaping stomach reduction in rodent (mg/kg-d) estimated by PK modeling. Animal BW set to study/sex-specific time-weighted average values for PK modeling. This explains the discrepancy in internal dose between male and female rats having the same external-dose LOAEL for ALT changes at 90 days.
[b]$BW^{3/4}$ scaling adjustment: mg/kg-d multiplied by $(BW_A/80)^{1/4}$). Animal BW set to study/sex-specific time-weighted average values for both $BW^{3/4}$ scaling and bioassay PK simulation.
[c]POD_{HED} in units of mg/kg-d Cr(VI) oral dose ingested by humans (lower 1% value of 20000 Monte Carlo PK simulations needed to achieve the internal dose POD). See Appendix C.1.5 for details.
[d]Data were amenable to BMD modeling with the highest dose omitted.

Consistent with EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), a series of five UFs were applied to the POD developed for each endpoint/study, specifically addressing the following areas of uncertainty: interspecies uncertainty (UF_A) to account for animal-to-human extrapolation, and consisting of equal parts representing pharmacokinetic and pharmacodynamic differences; intraspecies uncertainty (UF_H) to account for variation in susceptibility across the human population, and the possibility that the available data may not be representative of individuals who are most susceptible to the effect; LOAEL-to-NOAEL uncertainty (UF_L) to infer an exposure level where effects are not expected when a POD is based on a lowest-observed-adverse-effect level (LOAEL); subchronic-to-chronic uncertainty (UF_S) to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure, and to consider whether lifetime exposure would have effects at lower levels (e.g., for studies other than subchronic studies); and database uncertainty (UF_D) to account for database deficiencies if an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or life stage. An explanation of the five possible areas of uncertainty and variability follows:

- An intraspecies uncertainty factor, UF_H, of 3 was applied to account for variability and uncertainty in pharmacodynamic susceptibility in extrapolating to subgroups of the human population most sensitive to the health hazards of Cr(VI) (U.S. EPA, 2002). In the case of Cr(VI), the PODs were derived from studies in inbred animal strains and are not considered sufficiently representative of the exposure and dose-response of the most susceptible human subpopulations (see Section 3.3.1). In certain cases, the pharmacokinetic component of this factor may be replaced when a PK model is available that incorporates the best available information on variability in pharmacokinetic disposition in the human population (including sensitive populations). In the case of Cr(VI), a Monte Carlo analysis using PBPK modeling (see Appendix Section C.1.5) was applied to account for pharmacokinetic variability, and 3 was retained for pharmacodynamic variability.
- An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to all PODs to account for uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between rodents and humans. For all datasets used in this

assessment, a PBPK model or $BW^{3/4}$ scaling was used to convert doses in rodents to equivalent doses in humans (see rationale in Section 4.1.2.1—Human Extrapolation). This reduces pharmacokinetic uncertainty in extrapolating from the rodents to humans, but does not account for interspecies differences due to pharmacodynamics. An UF_A of 3 was applied to account for this remaining pharmacodynamic and any residual pharmacokinetic uncertainty not accounted for by the PBPK model.

- A subchronic-to-chronic uncertainty factor, UF_s, of 1 was applied to all endpoints from the chronic 2-year (lifetime) study in rodents (NTP, 2008) where exposure occurred for one year or more. For example, ALT changes in rats measured at one year (12 months) were assigned an UF_S of 1. An UF_S of 1 was applied to the developmental endpoint from NTP (1997), because exposure occurred during the critical window. An UF_s of 3 was applied to ALT changes from the 90-day study in rodents (NTP, 2007), and ALT changes reported at 3 months during the chronic NTP (2008) study. An UF_S = 3 (rather than 10) was applied to 90-day data for ALT because data collected at multiple time points from NTP (2008) showed that these effects did not increase in severity between 90 days and 1 year. A value of 3 was retained to account for the possibility that longer exposure may induce these effects at a lower exposures (U.S. EPA, 2002), even if the effects themselves do not increase in severity.
- A LOAEL-to-NOAEL uncertainty factor, UF_L, of 1 was applied to PODs based on either a NOAEL or a BMDL. An UF_L of 10 (rather than 3) was applied to PODs based on the LOAEL of ALT changes in rats observed from the 90-day study (NTP, 2007), because the magnitude of change from control at the lowest dose was very high (180% for males and 585% for females). These measurements were somewhat volatile (for example, the changes were typically very large, and the magnitude of changes varied greatly between studies, even among the NTP studies in the same species and sex which were conducted under very similar conditions). As a result, the higher UF_L was applied. Similarly, an UF_L of 10 was applied to the LOAELs of hyperplasia in the female mouse duodenum and chronic liver inflammation in female rats from NTP (2008) because responses were high (>20% extra risk) at the lowest dose. Thus, an UF_L of 10 was applied to all PODs that were based on a LOAEL.
- A database uncertainty factor, UF_D, value of 1 was applied for all endpoints. The toxicological database for oral exposure to Cr(VI) includes several occupational health studies, and subchronic and chronic toxicity studies in multiple laboratory species. The database also contains prenatal, multi-generational, and gestational oral studies in rodents.

Table 4-4 is a continuation of Table 4-3 and summarizes the application of UFs to each POD to derive a candidate value for each endpoint, preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative oral reference value for a specific hazard and subsequent overall RfD for Cr(VI).

Table 4-4. Effects and corresponding derivation of candidate values

Endpoint and Reference	POD_{HED} (mg/kg-day)	POD Type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
GI tract									
Mouse (M) hyperplasia (NTP, 2008)	0.0443	$BMDL_{10\%ER}$	3	3	1	1	1	10	4.43×10^{-3}
Mouse (F) hyperplasia (NTP, 2008)	0.0911	LOAEL	3	3	10	1	1	100	9.11×10^{-4}
Liver									
Rat (M) liver ALT (12 months) (NTP, 2008)	0.206	$BMDL_{1RD}$	3	3	1	1	1	10	0.0206
Rat (M) liver ALT (3 months) (NTP, 2008)	0.184	NOAEL	3	3	1	3	1	30	6.13×10^{-3}
Rat (M) liver ALT (90 days) (NTP, 2007)	0.203	LOAEL	3	3	10	3	1	300	6.77×10^{-4}
Rat (F) liver ALT (90 days) (NTP, 2007)	0.190	LOAEL	3	3	10	3	1	300	6.33×10^{-4}
Rat (F) liver chronic inflammation (2 years) (NTP, 2008)	0.0669	LOAEL	3	3	10	1	1	100	6.69×10^{-4}
Mouse (F) liver chronic inflammation (2 years) (NTP, 2008)	0.182	$BMDL_{10\%ER}$	3	3	1	1	1	10	0.0182
Rat (F) liver fatty change (2 years) (NTP, 2008)	0.0669	NOAEL	3	3	1	1	1	10	6.69×10^{-3}
Developmental									
Mouse (F) Decreased F1 postnatal growth (NTP, 1997)	0.700	NOAEL	3	3	1	1	1	10	0.0700

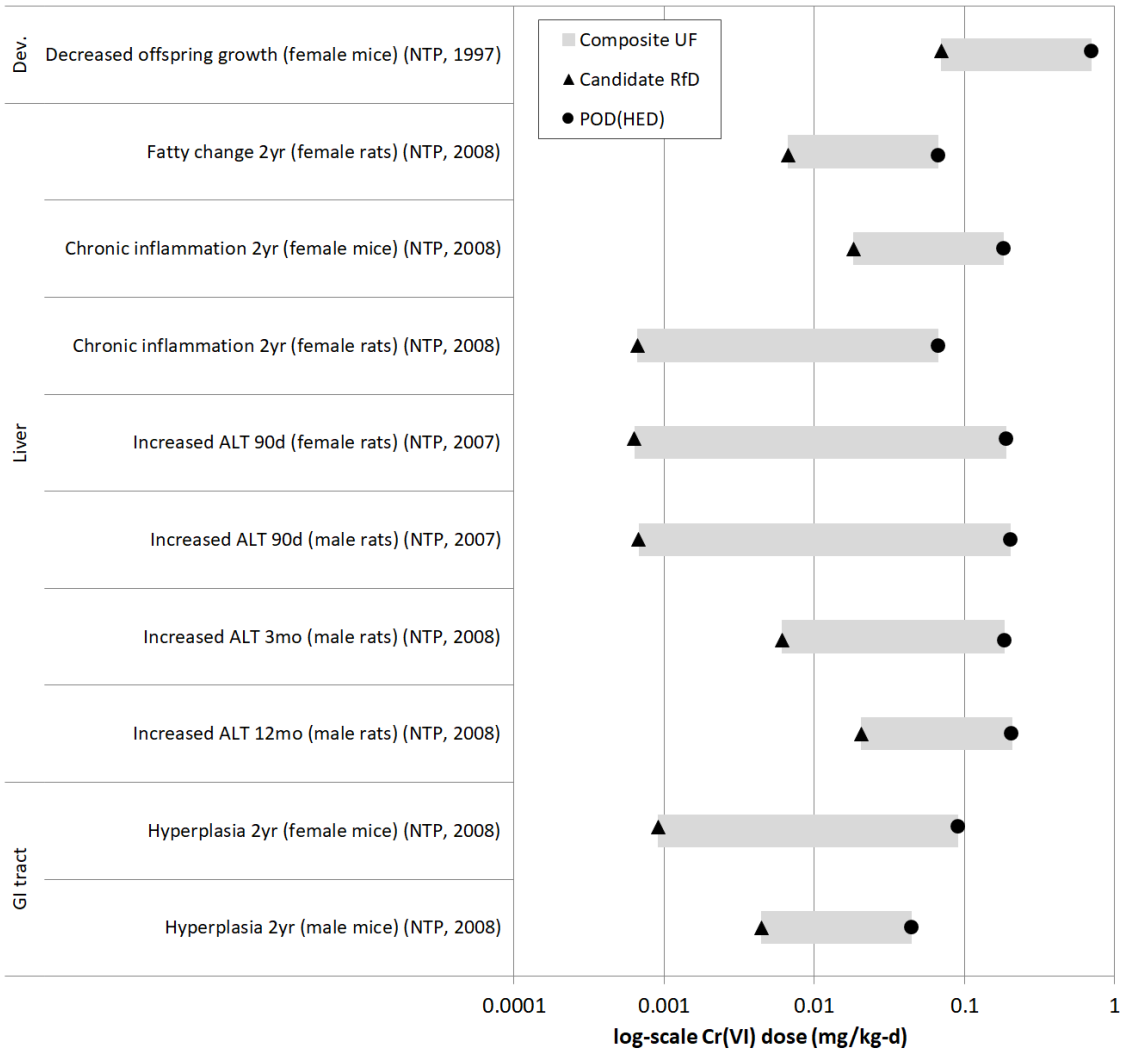

Figure 4-3. Candidate values with corresponding POD and composite UF.

4.1.4. Derivation of Organ/System-Specific Reference Doses

Table 4-5 distills the candidate values (candidate RfDs, or cRfDs) from Table 4-4 into a single value for each organ or system (organ-specific RfDs, or osRfDs). These organ or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Each candidate value was evaluated with respect to multiple considerations, including strength of evidence, basis of the POD (i.e., BMD vs. NOAEL vs. LOAEL), and dose-response model uncertainties. The confidence rating of each organ-specific RfD is based on three factors: the level of confidence in the primary study, the health effect database associated with that reference value, and the quantification of the POD.

4.1.4.1. *GI tract toxicity*

The organ/system-specific RfD for GI effects was based on the incidence of diffuse epithelial hyperplasia of the duodenum in female B6C3F1 mice reported in NTP (2008). The hyperplasia in the GI tract following oral exposures is considered to be representative of the constellation of histopathological observations that together result in a change in tissue function that is considered an adverse noncancer effect. An organ-specific RfD of 9×10^{-4} mg/kg-d (9.11×10^{-4} rounded to 9×10^{-4}) was derived. There is high confidence in this osRfD because it is based on chronic 2-year data from a *high confidence* study, and a strong dose-response was exhibited in both male and female mice. High confidence subchronic studies (click the HAWC link for study evaluation details) and mechanistic studies were supportive of these effects. Additionally, both male and female mice exhibited tumors in the same tissues (see Section 3.2.2), and these tumors exhibited a strong dose-response relationship.

4.1.4.2. *Hepatic toxicity*

The organ/system-specific RfD for hepatic effects was based on the lowest candidate toxicity value from the chronic data: chronic inflammation in female F344 rats reported in NTP (2008). Chronic hepatic inflammation can lead to fibrosis (Koyama and Brenner, 2017), and the candidate value is also protective of the other endpoints evaluated (fatty changes and chronic changes in clinical chemistry). An organ-specific RfD of 7×10^{-4} mg/kg-d (6.69×10^{-4} rounded to 7×10^{-4}) was derived. There is medium confidence in this osRfD. While it is based on a *high* confidence chronic study in rats and there are other subchronic data to support the liver endpoints, there were differences in the dose-response relationships between species and sexes. A lower organ-specific RfD confidence was assigned due to: 1) inconsistent responses across sex and species (e.g., histological changes were primarily seen in female rats and were less severe in male rats and mice), and 2) some uncertainty regarding the severity of the observed histological effects (specifically, the available high confidence studies did not observe a progression to more severe hepatic injury such as fibrosis or necrosis).

4.1.4.3. *Developmental toxicity*

The organ/system-specific RfD for developmental toxicity was based on the only candidate RfD: decreased F1 offspring postnatal growth from the continuous breeding study in BALBC mice (NTP, 1997). The organ-specific RfD was 0.07 mg/kg-d. There is low confidence in this osRfD. While it is based on a high confidence continuous breeding study and similar effects on decreased offspring growth observed in multiple other studies (see Section 3.2.9, click the HAWC link for study evaluation details), this effect only occurred in high dose groups where other toxicological effects (as indicated by the lower points of departure in this section) may be occurring. For example, female mice in the F0 generation (dams) were exposed to 11.6, 24.4, 50.6 mg/kg-d Cr(VI) (NTP, 1997). The decreased F1 offspring growth effect was observed at maternal dose of 24.4 mg/kg-d, which is a relatively high dose (NTP (2007) observed high incidence of stomach

ulcers in rats at approximately 20 mg/kg-d). Other studies in the database observing similar effects were lower confidence and used higher (or unknown) doses. A lower organ-specific RfD confidence was assigned due to: 1) a weak health effects database for this endpoint (most studies were rated *low confidence*), and 2) the possibility that other unknown toxicities could be affecting the animals at the high dose. Thus, there was lowered confidence due to the database of studies examining this endpoint, and lowered confidence in quantification of the POD.

Table 4-5. Organ/system-specific RfDs and proposed overall RfD for Cr(VI)

Effect	Basis	osRfD (mg/kg-day)	Exposure Description	Confidence
GI tract toxicity	Diffuse epithelial hyperplasia in small intestine (female mice)	9×10^{-4}	Chronic	High
Hepatic toxicity	Chronic inflammation (female rats)	7×10^{-4}	Chronic	Medium
Developmental toxicity	Decreased F1 offspring postnatal growth (mice)	0.07	Continuous breeding	Low
Overall RfD	GI tract effects	9×10^{-4}	Chronic	High

4.1.5. Selection of the Overall Reference Dose

Choice of the overall RfD involved consideration of both the level of certainty in the estimated organ/system-specific values, as well as the level of confidence in the observed effect(s). An overall confidence level was assigned to the RfD to reflect an interpretation regarding confidence in the collection of studies used to determine the hazard(s) and derive the RfD, the RfD calculation itself, as well as the overall completeness of the database on the potential health effects of hexavalent chromium exposure.

To estimate an exposure level below which noncancer effects from lifetime oral Cr(VI) exposure are not expected to occur, the osRfD for GI effects, 9×10^{-4} mg/kg-d, is selected as the overall RfD for Cr(VI). This was a *high* confidence value derived from chronic exposure data. The overall RfD is derived to be protective of all types of noncancer effects for lifetime exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002). While the organ-specific RfD for liver was slightly lower, that value was of lower confidence and the osRfD for GI effects is still protective of the other candidate values for liver that were considered for the osRfD (see Figure 4-3).

This value should be applied in general population risk assessments. However, decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific life stages of concern. For example, fluctuations in exposure levels that result in elevated exposures during various life stages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD.

4.1.6. Uncertainties in the Derivation of Reference Dose

The RfD was derived based on GI effects (diffuse epithelial hyperplasia in the duodenum) of female mice exposed to Cr(VI) in drinking water for two years (NTP, 2008). Some of the uncertainty considerations related to the RfD derivation are outlined below and in Section 3.3.

4.1.6.1. *Site concordance and human relevance*

The GI tract reference value was based on an effect observed in the small intestine of mice, however it is possible that the effect may be exhibited in different sections of the alimentary tract in the human (specifically, the oral cavity, esophagus, and stomach). Estimated Cr(VI) exposure to the stomach epithelium may be similar to exposure to the small intestine epithelium, since both would be strong functions of gastric pH, Cr(VI) concentration and reduction rate. There are differences in morphologies between the small intestine and stomach, which could potentially impact the tissue susceptibility. Effects in the rodent stomach only occurred at the high doses of the 90-day NTP (2007) study. Rodents exposed to Cr(VI) during the 2-year NTP (2008) study did not exhibit effects in the stomach.

Exposure to the oral cavity and esophagus occurs prior to Cr(VI) reduction in the stomach. However, no noncancer effects were observed in these tissues during the NTP (2008) or NTP (2007) bioassays (aside from mild salivary gland atrophy in rats during the 2-year study).

4.1.6.2. *Susceptible populations*

A significant fraction of the human population may be highly susceptible to Cr(VI)-induced effects in the GI tract due to high stomach pH. Individuals with hypochlorhydria (low stomach acid) have consistently high stomach pH that may exceed 8 (Feldman and Barnett, 1991). Less than 1% of the adult population may exhibit hypochlorhydria, whereas 10–20% of the elderly population (aged 65 and up) may exhibit this condition (Russell et al., 1993). For individuals without this medical condition, there is still high variability (Feldman and Barnett (1991) estimated that 5% of men may exhibit basal pH exceeding 5, and 5% of women may exhibit basal pH exceeding 6.8). Gut microbiota and gastric juice chemistry in individuals with high gastric pH may differ from those in the general population. It is not known how effective Cr(VI) can reduce to Cr(III) in this type of gastric environment. Data by Kirman et al. (2016), which included some groups with high stomach pH, were highly variable.

Individuals taking medication to treat gastroesophageal reflux disease (GERD), including calcium carbonate-based acid reducers and proton pump inhibitors, have an elevated stomach pH during treatment. This is known to be a significant fraction of the population since up to 20% of the population may be afflicted by GERD, and the gastric pH for these individuals may be above 4 throughout the day during successful treatment (Delshad et al., 2020; GBD 2017, 2020; Lin and Triadafilopoulos, 2015; Burdsall et al., 2013; Atanassoff et al., 1995) . A sensitivity analysis was performed on the human model (Appendix C.1.5), assuming a baseline stomach pH = 4 (as opposed to 1.3). It was found that for internal PODs above 0.001 mg/kg-d (which apply to all the PODs), the

current Monte Carlo approach (taking the lower 1% of 20,000 simulations of the standard population with baseline stomach pH = 1.3) was protective for the population with baseline pH = 4. For populations with baseline pH higher than 4, cRfDs derived using the pharmacokinetic approach would not be health-protective. Appendix D.3 contains cRfDs calculated by default approaches without adjustment for gastric reduction, which may be health-protective at low doses for the pH > 4 population (since those results implicitly assume gastric pH and reduction capacity in rodents and humans are equivalent).

Uncertainties related to extremely high gastric pH, as well as other conditions that could lead to pharmacokinetic susceptibility (*H. pylori* infection, gastric bypass, gastrectomy) cannot be accounted for quantitatively. High interindividual variation was observed in ex vivo data by Kirman et al. (2016), both in health individuals with high stomach pH, and individuals taking proton pump inhibitors. Additionally, no data are available studying Cr(VI) reduction in the gastric environments of children, toddlers, or infants. As a result, PBPK modeling was not performed for these groups.

4.1.6.3. *Rodent gastric modeling uncertainties*

Stomach reduction in the mouse may be impacted by a number of factors. Higher reduction efficiency may occur during the ingestion of a solid meal, since gastric emptying is delayed, and pH is decreased (for the mouse, glandular stomach pH is decreased by the fasted state, while the opposite is true for humans). However, this effect may be counter-acted by kinetics in the forestomach, which humans do not have. The forestomach may not follow the same fed/fasted pattern as the glandular stomach (Ward and Coates, 1987).

The rodent glandular stomach actively secretes digestive enzymes shortly before, during, and after a solid meal. The precise dynamics of gastric changes are uncertain, and the "well-mixed" PBPK model assumption may not be accurate due to ongoing food consumption. In addition, the rodent forestomach contents may have an elevated pH relative to the glandular stomach (Kohl et al., 2013; Browning et al., 1983; Kunstyr et al., 1976), and ingested drinking water passes through both of these stomach regions.

There are also uncertainties related to the pH-kinetic relationship. The dose-response analysis for this assessment applied rodent pH of greater than 4.0, setting pH to values at which the rodent ex vivo reduction experiments were performed. Prior to dilution with water, Proctor et al. (2012) estimated the rodent stomach pH to be approximately 4, but it was increased to approximately 4.5 after dilution with water for the experiments. The precise relationship between pH and reduction kinetics in the rodent at lower pH is uncertain, and therefore it was desirable to perform simulations assuming rodent pH of 4.0 or higher. If the true rodent stomach pH is lower, or if the reduction kinetics are faster than estimated by the current model, this would ultimately lead to a decreased RfD. On the other hand, the model already estimates a low percentage of Cr(VI) escaping the rodent stomach (5–10%). If the true percentage was lower than this, it would mean that a negligible amount of Cr(VI) enters the mouse small intestine following ingestion. It has been confirmed by multiple pharmacokinetic studies that Cr(VI) is absorbed systemically in rodents

following exposure via drinking water. Data by Kirman et al. (2012) show chromium concentrations in the duodenum increasing with a linear or supralinear relationship with respect to dose in mice exposed to Cr(VI) in drinking water for 90 days. Therefore, assuming that *in vivo* rodent gastric reduction occurs very effectively (i.e., 99% reduction) would not be consistent with the available pharmacokinetic data.

4.1.6.4. *Human gastric modeling uncertainties*

As with the rodent gastric system, there are uncertainties in modeling the human stomach. There exist complex gastric and intestinal kinetic models, and many of the parameters are highly variable (Paixão et al., 2018; Talattof and Amidon, 2018; Yu et al., 2017; Hens et al., 2014; Mudie et al., 2010; ICRP, 2006). While the PBPK model in this assessment adopts some parameters and concepts from literature, and incorporates Monte Carlo analysis, it may not account for all uncertainty and variability. Ex vivo data for Cr(VI) reduction in gastric juices show high interindividual variability De Flora et al. (2016); Kirman et al. (2016). Interindividual variability in gastric contents and microbiota likely introduces variation in Cr(VI) reduction. Variability in reduction kinetic parameters (with the exception of the reducing capacity parameter) was not incorporated into the model.

4.1.6.5. *Uncertainty in systemic pharmacokinetics*

The current approach uses a PBPK model of the stomach lumen to adjust the average daily oral Cr(VI) dose to account for detoxification in the stomach compartment. It does not explicitly model systemic whole-body pharmacokinetics. While whole-body PBPK models are available for Cr(VI), the uncertainties related to the systemic pharmacokinetics in rodents and humans are high, especially at low doses. However, most endpoints observed following oral ingestion were in or near the GI tract, and therefore may not require an accounting of systemic chromium. Cr(VI) which enters the intestinal lumen may expose the systems in which effects were observed (the small intestine, and the liver by first-pass effect) prior to distribution to systemic circulation. Reduction of Cr(VI) in the blood and other tissues is rapid, and this assessment neglects the impact that re-circulating Cr(VI) may have on the liver and small intestine. It is health-protective to assume that any unreduced Cr(VI) emptying into the human small intestine is absorbed.

For systemic effects, there is some residual pharmacokinetic uncertainty. The modeling does not take into account how much Cr(VI) may remain in the GI epithelium (or be reduced by the G.I. tissues, liver, and blood). This loss of Cr(VI) available to absorb into systemic tissues is neglected in both animals and humans.

4.1.6.6. *Uncertainty in dose-response modeling*

For the two lowest candidate RfDs (diffuse epithelial hyperplasia in female mice, and chronic liver inflammation in female rats from NTP (2008)), there was uncertainty related to the dose-response modeling.

As noted in Section 4.1.6, diffuse epithelial hyperplasia was not amenable to BMD modeling for female mice because there was too much uncertainty in estimating the BMDL. Estimates of the epithelial hyperplasia RfD from female mice using BMD modeling (without dropping doses) range from 7.95×10^{-5} mg/kg-d to 2.04×10^{-3} mg/kg-d (see Appendix D.1.1). The current value (derived by a LOAEL, which resulted in a higher uncertainty factor) falls within this range and differs by approximately 15% from the mean and median value of the three adequately fit models (1.06×10^{-3} mg/kg-d). If dropping the two highest doses and performing BMD modeling, the resulting RfD would be 2.6×10^{-3} mg/kg-d (and round to 3×10^{-3} mg/kg-d).

Similarly, chronic liver inflammation in female rats from NTP (2008) was not amenable to BMD modeling. Estimates of the cRfD for this endpoint range from 1.00×10^{-4} to 4.02×10^{-3} (see Appendix D.1.1). The current value (derived by a LOAEL, which resulted in a higher uncertainty factor) falls within this range and is about 2x lower than the mean and median values of the three adequately fit models (mean: 1.80×10^{-3} mg/kg-d, median: 1.28×10^{-3} mg/kg-d).

4.1.7. Confidence Statement

An overall confidence level of **High**, **Medium**, or **Low** was assigned to reflect the level of confidence in the study(ies) and hazard(s) used to derive the RfD, the overall database, and the RfD itself, as described in EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* §4.3.9.2 (U.S. EPA, 1994).

The confidence in the overall chronic RfD is **high**. The RfD is based on a *high confidence* chronic 2-year drinking water study by NTP (2008) which exposed rats and mice of both sexes to Cr(VI) as sodium dichromate dihydrate at drinking water concentrations from 5 mg/L to 180 mg/L (approximately 0.2 mg/kg-d to 10 mg/kg-d). Multiple *high confidence* subchronic studies also support these data (click the HAWC link for study evaluation details), and mechanistic studies support oxidative stress as a mechanism of Cr(VI) toxicity in a variety of tissues, including the GI tract. The organ-specific RfD for the liver is also supportive of the GI tract RfD, because the GI tract and liver are exposed on first-pass following oral ingestion (so both should get the highest internal dose). While the human database for Cr(VI) induced GI and liver toxicity was *indeterminate*, this did not warrant changing the overall confidence from *high*.

4.1.8. Previous IRIS Assessment: Oral Reference Dose

The previous RfD assessment for hexavalent chromium was completed in September 1998. The previous RfD was based on a NOAEL identified from a 1-year drinking water study in rats in which animals were exposed to hexavalent chromium (MacKenzie et al., 1958). MacKenzie et al. (1958) monitored body weight, gross external conditions, histopathology and blood chemistry and did not observe any effects at any level of treatment. A NOAEL of 2.5 mg/kg-day was identified. A composite uncertainty factor of 300 (10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for subchronic-to-chronic extrapolation) and a modifying factor of 3 (to

account for concerns raised by the epidemiology study of Zhang and Li (1987)) were applied to this POD to yield an oral RfD of 3×10^{-3} mg/kg-d.

4.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The reference concentration (RfC, expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with uncertainty factors generally applied to reflect limitations of the data used. As noted in Section 3.3.2, derivation of the RfC was limited to effects in the respiratory tract.

Upper respiratory toxicity in the form of nasal effects in humans has been determined previously (see Protocol Section 3.1.2, Appendix A), and a set of human studies were evaluated for data that may inform the quantitative dose-response analysis (this will be discussed in Section 4.2.1). Data suitable for candidate RfC derivation of upper respiratory effects were only available from human studies (and these were limited to effects in the nasal airways). Data from animals of effects in the upper respiratory tract (such as reported nosebleeds and other qualitative effect descriptions) were not considered due to the availability of quantitative dose-response data in humans.

Based on findings from inhalation studies in experimental animals and occupational studies in humans, evidence indicates that Cr(VI) is likely to cause lower respiratory toxicity in humans (see Section 3.2.1). Data suitable for candidate RfC derivation of lower respiratory effects were only available from animal studies. All human studies of these effects were *low* confidence and only provided information on associations (and did not provide dose-response data).

4.2.1. Identification of Studies for Dose-Response Analysis of Selected Effects

4.2.1.1. *Upper respiratory tract effects*

Effects in the nasal cavity of humans are well-established hazards of inhaled Cr(VI) exposure, and this review focused on data that may improve the quantitative dose-response analysis conducted in EPA's 1998 IRIS assessment (see Protocol Section 3.2, Appendix A). Quantitative animal data for effects in the upper respiratory tract were not available. Qualitative findings in rodents such as obstructive respiratory dyspnea (Glaser et al., 1990), or "peculiar sound during respiration" and periodic nose bleeds (Kim et al., 2004) were not considered for dose-response assessment due to the availability of human data. No other effects in the upper respiratory tract outside of the nasal cavity were identified during hazard identification (Section 3.2.1).

The epidemiological database for inhalation of Cr(VI) mainly consists of observational studies of workers exposed in occupational settings. Human studies were considered suitable for

dose-response analysis and toxicity values derivation if they met the criteria listed below. Furthermore, preference was given to studies with *medium* or *high* overall confidence ratings based on study evaluation and to studies with larger sample sizes and exposures in the lower range of human exposures, as these are most likely to represent the relationship between inhalation exposure to Cr(VI) and adverse effects in the general population.

The following considerations were made during evaluation of studies for derivation of inhalation toxicity values from human data:

- The study population must be exposed to Cr(VI) (as opposed to Cr(III)) based on air measurements or job history and industry
- Quantitative estimates relating exposure (or dose) to the core outcomes considered.
- Concentration of Cr(VI) in air must be measured at the study site
- Quality of measurements will depend on: type of sampling (personal, stationary, or both); frequency of sampling; sampling duration; number of samplers; sampling methods.
- Exposure to Cr(VI) for individuals or groups of individuals must be estimated with reasonable accuracy and precision in units of air concentration.
- If exposure is categorical, it must have corresponding air concentration estimates for each category.
- Exposure is not solely quantified in units of concentration in a biological sample such as urine or blood

The core outcomes for nasal effects in humans considered for evaluation of dose response included the following clinical outcomes diagnosed by a trained examiner (e.g., physician, otolaryngologist, or trained researcher): atrophy of the nasal mucosa, ulceration of the nasal mucosa or septum, perforation of the septum, and bleeding nasal septum. The development of these outcomes is highly specific to exposure to Cr(VI) and occurrence outside this exposure scenario is extremely rare. Consistent with this specificity of outcome, perforation of the septum has been known as "chrome hole" since the early days of chromium-related industries (including chromate production and electroplating (Bloomfield and Blum, 1928)). Furthermore, the presence of nasal pathologies considered here are occasionally used as supplemental information to confirm exposure to chromium in studies of non-nasal outcomes (Ciminera et al., 2016; Gibb et al., 2015; Machle and Gregorius, 1948). The specificity of this outcome to Cr(VI) exposure makes it ideal for the estimation of the dose-response relationship for noncancer effects in humans.

There were over 20 peer-reviewed studies of nasal effects that contained information related to endpoints in the nasal cavity, but these did not meet all criteria for dose-response analysis outlined above and were therefore not evaluated for study quality. There were also five non-peer-reviewed reports examining effects in the nasal cavity available from the National Institute for Occupational Safety and Health (NIOSH). These include Ceballos et al. (2017), Zey and Lucas (1985), Lucas (1976), Lucas and Kramkowski (1975), Cohen and Kramkowski (1973) and

Almaguer and Kramkowski (1983). Many of these studies did not have multiple exposure groups (either a referent or low/high concentration groups). Exposure and health effect data from these studies were only available for short time periods, and data were only collected after health effects were reported for the purpose of evaluating plant industrial hygiene practices (potentially leading to bias). As a result, most of these were excluded for dose-response consideration. Only data from Cohen and Kramkowski (1973) and its related peer-reviewed study (Cohen et al., 1974) were considered since this study contained a referent group. All studies excluded based on criteria above are listed at the bottom of Table 4-6, and detailed rationale for why each of these were not considered is provided in Appendix D.4 Table D-27.

Four peer-reviewed studies (some of which were associated with additional related studies containing exposure or study design information) initially met the criteria to be considered for toxicity value derivation and underwent formal study evaluation using HAWC. These were Gibb et al. (2000a), Lindberg and Hedenstierna (1983), Cohen et al. (1974), and Hanslian et al. (1967). All were conducted in occupational settings and the study populations were workers in either the chromate production or chrome electroplating industries. One study of 2,307 chromate production workers Gibb et al. (2000a), though retrospective in design, utilized company records of air concentration data, individual job and task data, and data from regular medical examinations, to construct a dataset that included individual exposure estimates for each worker as well as the time from baseline exposure to the incident event of the health outcome (see Table 4-23 in Section 4.4.5). The other three studies (Lindberg and Hedenstierna, 1983; Cohen et al., 1974; Hanslian et al., 1967) were cross-sectional in design and were conducted in smaller study populations composed of chrome electroplating workers. The populations were adults, and the largest cohort (Gibb et al. (2000a), which had a population size of 2307) only had male workers.

Three studies were classified as *medium* confidence (Gibb et al., 2000a; Lindberg and Hedenstierna, 1983; Cohen et al., 1974), and one study was *low* confidence (Hanslian et al., 1967). Because of the availability of *medium* confidence studies, data from Hanslian et al. (1967) were no longer considered for dose-response. In addition to the usual factors considered during study evaluation, diagnosis of nasal outcomes after physical examination of the nasal cavity by a trained examiner was considered when determining confidence ratings for nasal effects studies. Additional study details, including the reported endpoint data, are provided in Table 4-7.

Table 4-6. Evaluation of epidemiology studies on hexavalent chromium and nasal effects. Click to see interactive data graphic for rating rationales.

			Study evaluation							
	Reference	Study description	Exposure	Outcome	Selection	Confounding	Analysis	Sensitivity	Sel. reporting	Overall confidence
Included	Gibb et al. (2000a) related: Gibb et al. (2015) Braver et al. (1985); Hayes et al. (1979)	Occupational longitudinal study. Male workers in a chromate production plant in Baltimore, MD (n = 2307).	A	A	A	A	A	G	A	MED
	Lindberg and Hedenstierna (1983)	Cross-sectional study. Male and female employees in chrome-plating industry (n = 104). Office employees (n = 19) as reference group	A	A	A	A	A	A	A	MED
	Cohen et al. (1974) Related: Cohen and Kramkowski (1973)	Cross-sectional study. White male and female electroplating workers in nickel-chrome department (n = 37) Randomly-chosen workers employed in other areas of the plant not significantly exposed to chromic acid as reference group (n = 15)	A	G	A	A	A	A	A	MED
Excluded	Hanslian et al. (1967)	Cross-sectional study. Male and female chrome-plating workers (n = 77). 53 working directly with baths, 23 working directly with chromium. No reference group.	D	A	D	A	A	A	A	LOW
Not suitable*	Almaguer and Kramkowski (1983), Armienta-Hernández and Rodríguez-Castillo (1995), Bloomfield and Blum (1928), Ceballos et al. (2019, 2017), Dornan (1981), Elhosary et al. (2014), Fagliano et al. (1997), Gomes (1972), Horiguchi et al. (1990), Huvinen et al. (2002a), Kleinfeld and Rosso (1965), Kitamura et al. (2003), Korallus et al. (1982), Lee and Goh (1988), Lin et al. (1994), Lucas et al. (1976; 1975), Machle and Gregorius (1948), Mancuso (1951), PHS (1953), Royle (1975), Singhal et al. (2015), Sorhan et al. (1998; 1987), Vigliani and Zurlo (1955), Wang et al. (1994), Yuan et al. (2016), Zey and Lucas (1985)								

G = good; A = adequate; P = poor.

*Studies that may have contained data for effects in the nasal cavity, but were determined not to meet PECO within the scope of derivation of nasal toxicity values, or were not suitable for dose-response analysis for other reasons. Rationale for excluding individual studies is available in Appendix D4 Table D-27.

Table 4-7. Dose-response data for effects in the nasal cavity of humans (*medium* confidence studies)

Study	Exposure	Conf	Result Format	Effects		
Lindberg and Hedenstierna (1983)	Chrome plating		Number of cases	**Ulceration** **8-hr mean air µg Cr(VI)/m³** **Group n cases (%)** ≤1.9 19 0 2–20 24 8 (33) **Highest air µg Cr(VI)/m³** **Group n cases** 0.2–1.2 10 0 2.5–11 12 0 20–46 14 7 (50)	**Atrophy** **8-hr mean air µg Cr(VI)/m³** **Group n cases (%)** ≤1.9 19 4 (21) 2–20 24 8 (33) **Highest air µg Cr(VI)/m³** **Group n cases** 0.2–1.2 10 1 (10) 2.5–11 12 8 (67) 20–46 14 0	**Perforation only*** **8-hr mean air µg Cr(VI)/m³** **Group n cases** ≤1.9 19 0 2–20 24 3 (13) **Highest air µg Cr(VI)/m³** **Group n cases** 0.2–1.2 10 0 2.5–11 12 0 20–46 14 3 (21) * 2 w/ulceration also had perforation (total w/ perforation = 5)
Gibb et al. (2000a)	Chromate production		Cumulative incidence (%) (n = 2307), onset time, and relative risk (ulceration only)	**Ulcerated nasal septum** **Effect:** 62.9% **Mean (median) exposure:** 0.054 (0.020) mg CrO_3/m³ or 28 (10) µg Cr(VI)/m³ **Mean (median) time on job (days) from date first hired to date of first diagnosis:** 86 (22)	**Perforated nasal septum** **Effect:** 17.3% **Mean (median) exposure:** 0.063 (0.021) mg CrO_3/m³ or 33 (11) µg Cr(VI)/m³ **Mean (median) time on job (days) from date first hired to date of first diagnosis:** 313 (172)	**Ulcerated septum relative risk** Adjusted relative risk for a 0.1 mg CrO_3/m³ increase (in ambient air) = 1.2 (by Cox proportional hazards model adjusted for calendar year at hire and age at hire, p = 0.0001).
Cohen et al. (1974)	Chrome plating		Prevalence (%) (with grading by severity)	**Nasal ulceration parameter cases, number (%)**		**non-exposed (n = 15)** **exposed (n = 37)**
				nasal mucosa (grade 0)		14 (93) 2 (5)
				shallow erosion of septal mucosa (grade 1)		0 8 (22)
				ulceration and crusting of septal mucosa (grade 2)		0 12 (32)
				avascular, scarified areas of septal mucosa w/o erosion or ulceration (grade 3)		0 11 (30)
				perforation of septal mucosa (grade 4)		1 (7) 4 (11)
				Exposed group area breathing zone: mean = 2.9 (ND–9.1) µg Cr(VI)/m³ **Referent area breathing zone:** 0.3 (0.1–0.4) µg Cr(VI)/m³		

1 mg CrO_3 = 0.52 mg Cr(VI).

4.2.1.2. *Lower respiratory tract effects*

The inhalation animal toxicological database for Cr(VI) consists of studies with chronic, subchronic, and/or acute data. Many of these studies analyzed similar or identical toxicological endpoints, particularly for the respiratory system. Within the endpoint-specific databases for hazard identification, a subset of these studies were considered for toxicity value derivation based on factors outlined in Section 4.1.1. Preference was given to studies with larger sample sizes and low concentrations, to facilitate extrapolation to levels typical of environmental human exposure (U.S. EPA, 2012b). For inhalation studies of particulates, studies that provided measures of particle size and distribution were preferred. Because of the availability of studies that were rated *medium* confidence for lower respiratory tract endpoints, *low* confidence studies were not considered for cRfC derivation. An outline of the process used to select candidate animal datasets for dose-response analysis and cRfC derivation is provided in Figure 4-4.

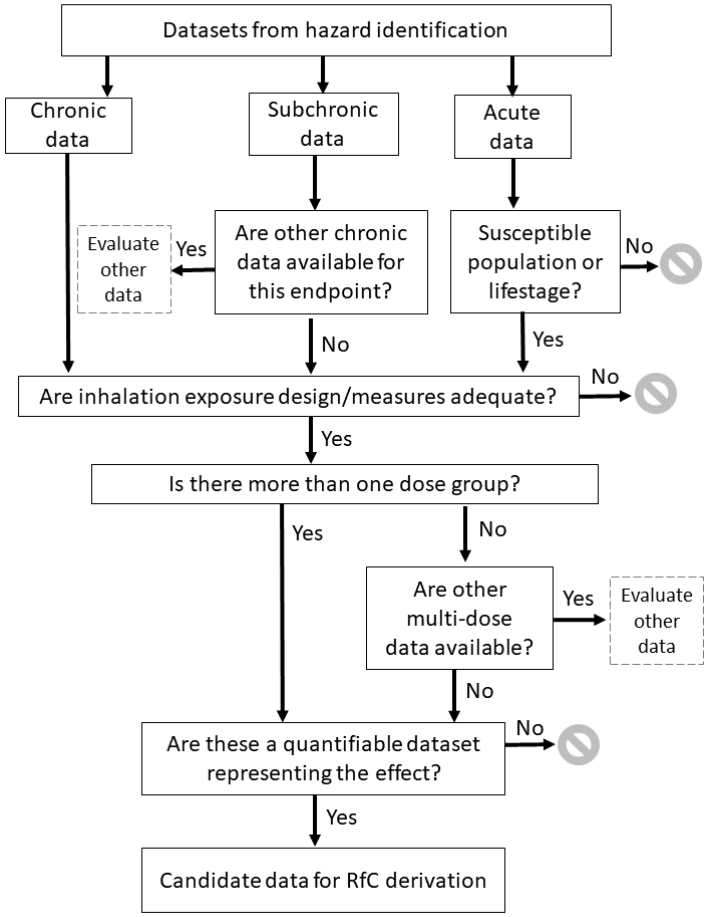

Figure 4-4. Evaluation of animal studies from the Cr(VI) hazard identification for derivation of toxicity values. *Low* confidence studies were not considered.

Table 4-8. Design features of inhalation studies that examined effects in animals

Study reference	Species/ strain and sex	Exposure duration	Dose groups[a]	Animals/ group	Chemical and particle size	Concentration range (mg/m^3 Cr[VI])
Glaser et al. (1990)	Wistar Rat, Male	30/90 days (22 hr/day, 7 d/wk)	4	10	Sodium dichromate MMAD 0.28 (±1.63) µm bottom two dose groups 0.39 (±1.72) µm high groups	0.05–0.4
Glaser et al. (1985)	Wistar Rat, Male	28/90 days (22 hr/day, 7 d/wk)	3	10	Sodium dichromate MMD 0.2 (±1.5) µm	0.025–0.2
Johansson (1986b; 1986a)	Rabbit, Male	4–6 weeks (inexact), 6 hr/day, 5 d/wk	1	8	Sodium dichromate MMAD 1 µm (approx.)	0.9
Cohen et al. (2003)	F344 Rat, Male	48 weeks, 5 hr/day, 5 d/wk	1	30	Calcium chromate MMAD 0.6 (±1.7) µm	0.36
Kim et al. (2004)	Sprague-Dawley Rat, Male	90 days, 6 hr/day, 5 d/wk	3	5	Chromium trioxide (size not reported)	0.2–1.25

[a]Number does not include control group

Table 4-4 outlines the inhalation studies rated *medium* or higher confidence for respiratory tract endpoints (all were rated *medium* confidence for lung histopathology cellular responses; see Section 3.2.1). Of the studies listed in Table 4-8 the Glaser et al. (1990; 1985) studies were preferred for cRfC derivation due to the number of exposure groups, sample sizes, and reporting of endpoints, methods, and particle sizes. Kim et al. (2004) did not report quantitative data for effects or chromium particle size, and effects, and Johansson (1986b; 1986a) and Cohen et al. (2003) only used a single high exposure group.

Lung histiocytosis, bronchioalveolar hyperplasia, and increased total protein and albumin in BAL fluid were observed by Glaser et al. (1990) after 90 days of exposure, and these measures remained slightly elevated after a 30-day recovery period (see Section 3.2.1). Although lactate dehydrogenase (LDH) in BAL fluid returned to normal following the 30-day recovery period, LDH is considered a sensitive indicator of cellular injury (Henderson et al., 1985), and there was a clear dose-response relationship. Dose-response data from Glaser et al. (1990) following 90 days of exposure (with and without the 30-day recovery period) are presented in Figures 4-5 and 4-6. Because histopathological and cellular changes occurred together, and in combination with serum biomarkers indicating an inflammatory response (Nikula et al., 2014), all exposure levels were considered to have induced adverse responses.

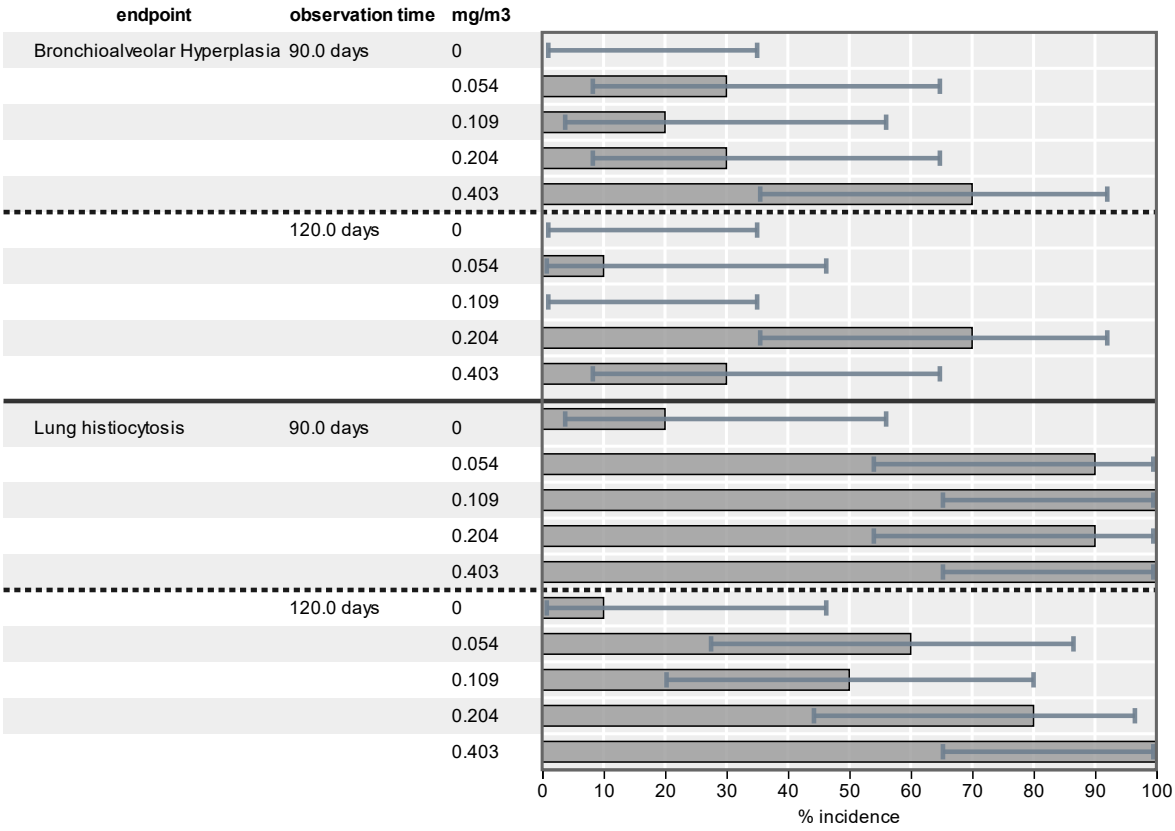

Figure 4-5. Dose-response relationship for lung histopathological in male rats using data from Glaser et al. (1990). Data are for 90-day observation time immediately following exposure, and 120-day observation time (90 days of exposure followed by a 30-day period of no exposure). N = 10/group. Click here for interactive graphic.

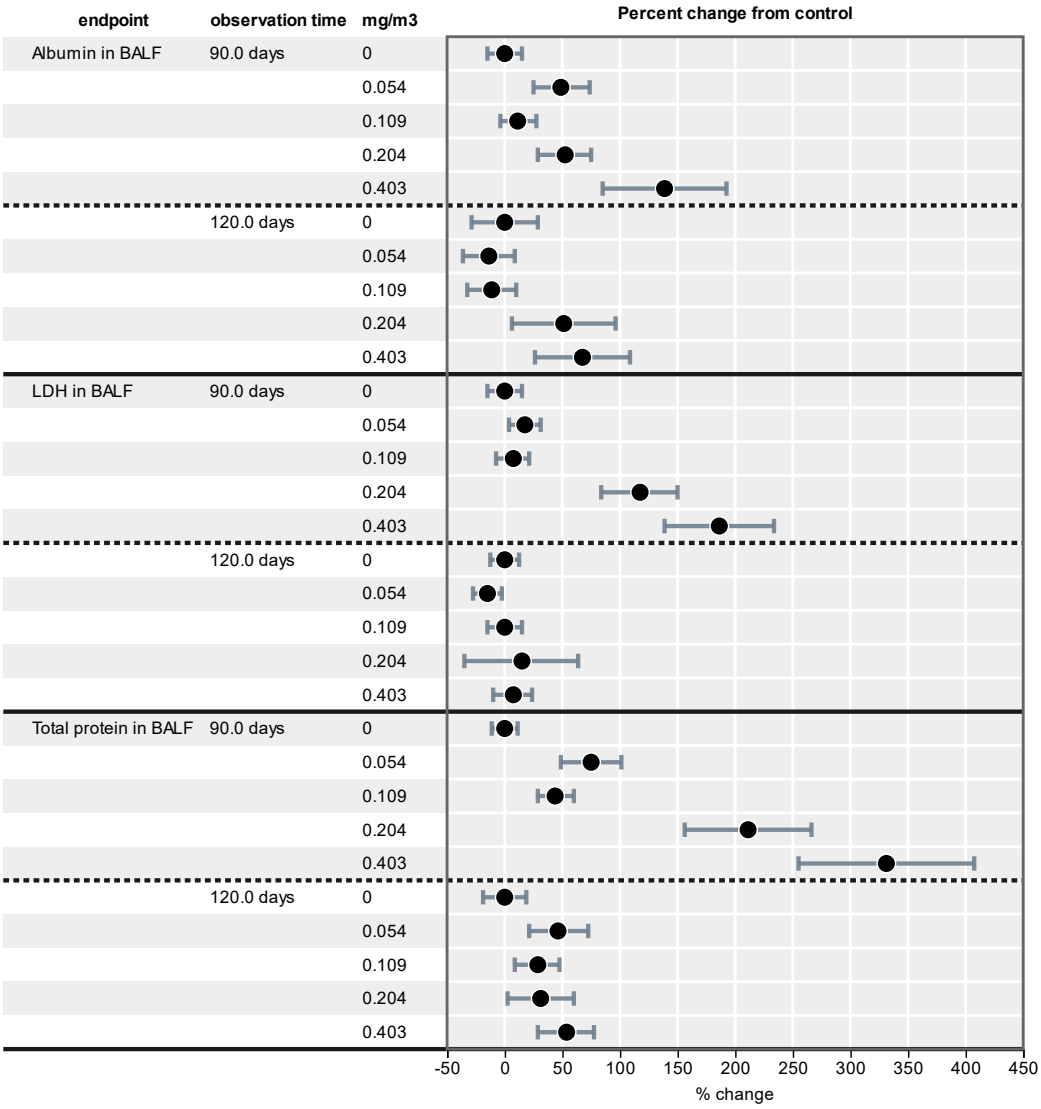

Figure 4-6. Dose-response relationship for selected endpoints in male rats using data from Glaser et al. (1990). Data (± 95% confidence interval) are for 90-day observation time immediately following exposure, and 120-day observation time (90 days of exposure followed by a 30-day period of no exposure). N = 10/group. Click here for interactive graphic.

The endpoints and datasets used for dose-response of lower respiratory tract effects were:

- BAL fluid measurements of total protein, albumin, and LDH from Glaser et al. (1990) at 90 days
- Lung histopathological findings of histiocytosis and bronchioalveolar hyperplasia Glaser et al. (1990) at 90 days

These endpoints were preferred because they are the most direct and sensitive indicators of cellular lung injury (Nikula et al., 2014; Henderson et al., 1985).

4.2.1.3. *Other effects*

Inhalation data for effects outside the respiratory system are limited. The only animal inhalation studies rating *medium* confidence for endpoints outside the respiratory tract that were determined to be a hazard in Section 3.2 were Kim et al. (2004) (liver weight and clinical chemistry) and Glaser et al. (1985) (liver histopathology). These studies did not observe effects in the liver following inhalation exposure. All human inhalation studies of outcomes outside the respiratory tract were rated *low* confidence for those domains. As a result, cRfCs were not derived for effects outside of the respiratory tract.

4.2.2. Methods of Analysis

4.2.2.1. *Analysis of animal data*

Animal data by Glaser et al. (1990) were used to derive candidate RfCs of lower respiratory tract effects. As noted earlier, the candidate endpoints were 1) BAL fluid measurements of total protein, albumin, and LDH; and 2) Lung histopathological findings of histiocytosis and bronchio-alveolar hyperplasia.

Biologically based dose-response models are not available for respiratory effects of Cr(VI). In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, EPA evaluated dose-response information with the models available in EPA's Benchmark Dose Software (BMDS, Version 3.2). However, data of lung histiocytosis, and LDH, albumin, and total protein in BAL fluid at the 90-day observation from the Glaser et al. (1990) study in rats were not amenable to BMD modeling (see Appendix Section D.2.1 for details). As a result, no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect levels (LOAEL) approaches were used for these effects. Because the lung histopathological changes and cellular responses in BAL fluid occurred together at the lowest exposure level, and in combination with serum biomarkers indicating an inflammatory response (Nikula et al., 2014), all exposure levels were considered to have induced adverse responses. Therefore, a LOAEL of 0.054 mg/m^3 was chosen as the POD for these endpoints. Bronchioalveolar hyperplasia was amenable to BMD modeling, and a BMR of 1 standard deviation[66] change from the control mean was applied.

Animal-to-human extrapolation

In accordance with EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), duration adjustments and dosimetric adjustment factors (DAFs) were used for extrapolating the selected/candidate PODs from animals

[66]As noted in Section 4.1.2, when no biological information is readily available that allows for determining a minimally biological significant response, the BMD Technical Guidance (U.S. EPA, 2012b) recommends a BMR based on one standard deviation (SD).

to humans in order to calculate human equivalent concentrations (HECs). Because the RfC is intended to apply to continuous lifetime exposures for humans (U.S. EPA, 1994), a duration adjustment was made to convert study-specific rodent bioassay exposure regimens to continuous exposures. Next, a dosimetric adjustment factor was applied to account for differences in particle lung dosimetry between species. Unlike for the RfD, extracellular reduction of Cr(VI) to Cr(III) was assumed negligible for the inhalation route of exposure, and no additional dosimetric factors were applied for pharmacokinetics.

The PODs identified from Glaser et al. (1990) were adjusted to account for discontinuous daily exposure regimens as follows:

$$POD_{ADJ} = POD \times (\text{hours exposed per day}/24 \text{ hours}) \times (\text{days exposed per week}/7 \text{ days})$$

Where POD is the external exposure concentration rodent POD (mg/m^3, determined by dose-response modeling of rodent data or from the study NOAEL or LOAEL) and POD_{ADJ} is the duration-adjusted experimental exposure concentration (mg/m^3).

Next, the POD_{HEC} was calculated from the POD_{ADJ} by multiplying by a DAF, which in this case was the regional deposited dose ratio ($RDDR_r$) for respiratory tract region *r* of interest as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

$$POD_{HEC} = POD_{ADJ} \times RDDR_R$$

The $RDDR_r$ can be calculated based on the physiology and respiratory parameters of rodents and humans, and predicted fractional deposition in each respiratory tract region for each species:

$$RDDR_r = \frac{(SA_r)_H}{(SA_r)_A} \times \frac{(V_E)_A}{(V_E)_H} \times \frac{(F_r)_A}{(F_r)_H}$$

where:

SA_r = surface area of respiratory tract region r (m^2 or cm^2)

V_E = ventilation rate (L/minute)

F_r = fractional deposition in respiratory tract region r

Since most effects in the BAL fluid may be indicative of effects due to deposition in the entire lung (with the exception of the upper airways), the total of the pulmonary (PU) and tracheobronchial (TB) surface areas and fractional depositions in these regions were used to calculate an $RDDR_{TB+PU}$:

$$RDDR_{TB+PU} = \frac{(SA_{TB+PU})_H}{(SA_{TB+PU})_A} \times \frac{(V_E)_A}{(V_E)_H} \times \frac{(F_{TB+PU})_A}{(F_{TB+PU})_H}$$

The factor $RDDR_{TB+PU}$ was employed for all BAL fluid endpoints (except albumin) because these effects were believed to be induced by exposure in the conducting airways and deep lung. For albumin in BAL fluid, $RDDR_{PU}$ was applied, because this effect is believed to be induced by exposure in the deep lung only.

Fractional depositions in the pulmonary region (F_{PU}) and tracheobronchial region (F_{TB}) for both rats and humans were calculated using the Multi-Path Particle Dosimetry (MPPD) model version 2.11[67], a computational model that can be used for estimating airway particle deposition and clearance (ARA (2009)).

For the model runs, the Yeh-Schum 5-lobe model was used for the human and the asymmetric multiple path model was used for the rat (see Appendix D.1.3 and D.1.4 for MPPD model outputs). Both models were run under nasal breathing scenarios with the inhalability adjustment selected. The aerosol Cr(VI) concentrations reported by Glaser et al. (1990) were converted to aerosol sodium dichromate concentrations by molecular weight conversion (see Appendix D.1.3). It was determined that aerosol concentration did not affect the predicted fractional lung depositions (human F_r values were identical if aerosol concentration was set to either 1 or 136 mg/m^3). Thus, the aerosol concentration at the lowest Cr(VI) concentration was applied for rodent-human extrapolation (reported concentration of 54 mg/m^3 Cr(VI) is equivalent to 136 mg/m^3 sodium dichromate aerosol). Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) reported by Glaser et al. (1990) varied slightly with concentration, however this had a negligible effect on the RDDR (see Appendix Section D.1.3). For MPPD simulations, the particle MMAD ± GSD (0.28 ± 1.63 μm), which reported for the lower Cr(VI) concentrations, was applied. The density of sodium dichromate was input as 2.52 g/cm^3.

The inhalation parameters used for the rat were: breathing frequency, 102 per minute (default); tidal volume, 2.1 mL (default); V_E, 0.214 L/minute (calculated); functional residual capacity, 4 mL (default); and upper respiratory tract volume, 0.42 mL (default). Adult human lung physiology was: functional residual capacity, 3,300 mL (default); and upper respiratory tract volume, 50 mL (default). Since human breathing frequency and tidal volume have a significant impact on the estimated F_r, and these parameters are a strong function of human activity, multiple different scenarios were simulated: resting, light work, heavy work, and maximal work. Values defined by EPA's *Exposure Factors Handbook* (U.S. EPA, 2011a) are contained in Appendix Table D-19, and range from 40 breaths/min at a tidal volume of 3050 mL (maximal work) to 12 breaths/min at a tidal volume of 500 mL (resting). All other parameters (rodent and human) were set to the default MPPD software values (see Appendix D.1.3).

For the human, regional-specific surface areas for the respiratory tract (used as normalizing factors) were 200 cm^2 for extrathoracic (ET), 3200 cm^2 for tracheobronchial (TB), and 54 m^2 for

[67]EPA has since released newer version of the model. The differences in RDDR between MPPD v2.11 (released by Applied Research Associates) and MPPD v1.01 (released by EPA) are less than 10% (see Appendix D.1.3).

pulmonary (PU) (U.S. EPA, 1994). For the rat, respiratory tract surface areas were 15 cm^2 for ET, 22.5 cm^2 for TB, and 0.34 m^2 for PU (U.S. EPA, 1994). The calculated RDDR values for TB/PU regions ranged from 2.12/7.00 (resting scenario) to 0.12/0.47 (maximal work scenario). Since the maximal and heavy work scenarios would not be representative of average daily lifetime inhalation rates and volumes, the RDDR values were taken to be the average of the mean adult resting and mean light work RDDRs. Values of RDDR were calculated as:

$RDDR_{PU}$: 3.435

$RDDR_{TB+PU}$: 2.685

Table 4-9 summarizes the sequence of calculations leading to the derivation of a human-equivalent point of departure for each data set discussed above.

Table 4-9. Summary of derivation of points of departure following inhalation exposure to hexavalent chromium. Data for male Wistar rats from Glaser et al. (1990)

Endpoint	% extra risk at LOAEL (mg/m^3)[a]	LOAEL or BMD (mg/m^3)	BMDL	POD_{ADJ} (mg/m^3)	RDDR	POD_{HEC} (mg/m^3)
Histopathology: histiocytosis	87.5%	0.054	N/A	0.0495	2.685 (TB+PU)	0.133
Histopathology: bronchioalveolar hyperplasia	30%	BMD_{1SD} = 0.0294[b]	$BMDL_{1SD}$ = 0.0168	0.0154	2.685 (TB+PU)	0.0413
Cell responses: LDH in BALF	17%	0.054	N/A	0.0495	2.685 (TB+PU)	0.133
Cell responses: Albumin in BALF	49%	0.054	N/A	0.0495	3.435 (PU)	0.170
Cell responses: Total protein in BALF	75%	0.054	N/A	0.0495	2.685 (TB+PU)	0.133

[a]%ER = (% incidence at LOAEL − % incidence at control)/(100 − % incidence at control) × 100
POD_{ADJ} = (BMCL or NOAEL or LOAEL) × (22/24) × (7/7), since rodents in the Glaser et al. studies were unexposed for 2 hours each day.
PU+TB: $POD_{HEC} = (POD_{ADJ}) \times RDDR_{TB+PU} = (POD_{ADJ}) \times 2.685$.
PU: $POD_{HEC} = (POD_{ADJ}) \times RDDR_{PU} = (POD_{ADJ}) \times 3.435$.
[b] Log-logistic model selected

4.2.2.2. *Analysis of human data*

Human data by Gibb et al. (2000a), Lindberg and Hedenstierna (1983), and Cohen et al. (1974) were used to derive candidate RfCs of upper respiratory tract effects. However, these effects could not be modeled by Benchmark Dose Software (BMDS) models or other specialized models. As noted in the analysis of nasal effects by OSHA (2006), the available human data were insufficient to relate exposures and incidence. Studies either did not have the proper study design for a quantitative analysis, or lacked short-term airborne Cr(VI) exposure data over an entire employment period (OSHA, 2006). Because none of the available studies provided data for a no-observed-adverse-effect-level (NOAEL), PODs were derived using lowest-observed-adverse-effect-levels (LOAELs). How these uncertainties were accounted for in the quantitative derivation of the cRfC are described later in this section.

The adjustment factors to account for differences between occupational exposures and non-occupational exposure follow EPA guidelines (U.S. EPA, 2009) that acknowledges there are differences in breathing rates between workers (10 m^3 per 8-hour day) and non-workers (20 m^3 per 24-hour day) and that workers are exposed 240 days per year while non-workers are exposed 365 days per year (U.S. EPA, 2016c, 2014e, 2012d, 2011d). If workplace exposure is assumed to occur 240 workdays/year:

$$LOAEL_{HEC} = LOAEL\ (\mu g/m^3) \times (VEho/VEh) \times 240\ days / 365\ days$$

Where:

$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to an ambient human equivalent concentration;

LOAEL = occupational exposure level (time-weighted average);

VEho = human occupational default minute volume (10 m^3/8 h); and

VEh = human ambient default minute volume (20 m^3/24 h).

Table 4-10. Summary of derivation of points of departure following human inhalation exposure to hexavalent chromium

Study	POD rationale	Notes and conversions	LOAEL ($\mu g/m^3$)	% incidence at LOAEL	POD HEC ($\mu g/m^3$)
Lindberg and Hedenstierna (1983)	Ulceration of the nasal septum. The midpoint concentration for the <2 and 2–20 µg Cr(VI)/m^3 groups. There is high uncertainty in the exposure concentrations.	Table 3	10	33%	3.3
Gibb et al. (2000a)	Ulceration of the nasal septum. The **median** exposure at first diagnosed nasal ulceration.	Table 1 20 µg CrO_3/m^3 = 10.4 µg Cr(VI)/m^3	10.4	63%	3.4
Gibb et al. (2000a)	Ulceration of the nasal septum. The **mean** exposure at first diagnosed nasal ulceration	Table 1 54 µg CrO_3/m^3 = 28 µg Cr(VI)/m^3	28	63%	9.2
Cohen et al. (1974) (related study: Cohen and Kramkowski (1973))	Ulceration of the nasal septum. Mean air concentration for exposed groups.	Table 6 0.0029 mg Cr(VI)/m^3 (2.9 µg Cr(VI)/m^3)	2.9	32%	0.95

Exposure adjustment for all study concentrations to obtain POD HEC used the following occupational/non-occupational factor: (10/20) × (240/365)

For ulceration of the nasal septum from Gibb et al. (2000a), the mean exposure concentration was over 2x the median concentration. This is an indication that the data are skewed, and that the median is a better estimate of the central tendency. As a result, the median result was preferred to the mean for this dataset.

4.2.3. Derivation of Candidate Values

The reference concentration (RfC) is the inhalation concentration likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime (U.S. EPA, 1994).

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002); Section 4.4.5], five possible areas of uncertainty and variability were considered. An explanation of the five possible areas of uncertainty and variability follows.

For PODs derived using either animal (lower respiratory) or human (nasal effect) data:

- A database deficiencies uncertainty factor, UF_D, value of 3 was applied. A value of less than 10 was applied because respiratory tract effects of inhaled Cr(VI) are considered portal-of-entry effects, and are therefore likely to be amongst the most sensitive based on current understanding of pharmacokinetics and mechanisms following inhalation. A value of $UF_D = 3$ (as opposed to $UF_D = 1$) was applied because many of the inhalation studies were *low* confidence (particularly for noncancer effects outside the portals of entry) and limited in scope (working-age and mostly male humans, and only male rodents). Due to pharmacokinetic differences from oral exposure (Cr(VI) is detoxified in the gut and liver on first-pass), the stronger oral database could not be used to inform the UF_D for inhalation effects.

For animal-derived PODs using data for lower respiratory effects from Glaser et al. (1990):

- A subchronic-to-chronic uncertainty factor, UF_S, of 3 was incorporated to account for the less-than-lifetime exposure in Glaser et al. (1990) (which was a 90-day study). A value of 3 accounts for the possibility that longer exposure may induce effects at a lower exposures (U.S. EPA, 2002).
- An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to account for residual uncertainty in the extrapolation from laboratory animals to humans in the absence of information to characterize pharmacodynamic differences between rats and humans after inhalation exposure to Cr(VI). This value is adopted when an adjustment from animal to a human equivalent concentration has been performed as described in EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). For these animal endpoints, an RDDR factor was used to estimate a human equivalent concentration from animal data.
- A LOAEL-to-NOAEL uncertainty factor, UF_L, of 3 was applied to LOAELs that were based on lung cellular and histopathological responses in BAL fluid. A value of less than 10 was applied because these responses were highly sensitive indicators of lung injury and inflammation (Nikula et al., 2014; Henderson et al., 1985). Additionally, effects began to resolve after a short recovery time (see Figures 4-5 and 4-6). Considering these characteristics, the changes were interpreted to approximate adverse responses, albeit with some residual uncertainty, which do not support application of a $UF_L = 10$.
- An intraspecies uncertainty factor, UF_H, of 10 was applied to account for variability and uncertainty in pharmacokinetic and pharmacodynamic susceptibility within the human population. The PODs were derived from studies in inbred animal strains, and data were

only available for males. This is not considered sufficiently representative of the exposure and dose-response of the most susceptible human subpopulations. In the case of inhaled Cr(VI), insufficient information is available to quantitatively estimate variability in human susceptibility; therefore, the full value for the intraspecies UF was retained.

For human-derived PODs using occupational data for effects in the nasal cavity:

- An interspecies uncertainty factor, UF_A, of 1 was applied because results were derived from studies in humans.
- A LOAEL-to-NOAEL uncertainty factor, UF_L, of 10 was applied because this endpoint had a high incidence at the lowest concentration across multiple studies. As a result, there was higher uncertainty in the exposure-response relationship at lower concentrations.
- An intraspecies uncertainty factor, UF_H, of 3 to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. The populations evaluated were mostly adult male workers, which is not representative of individuals who may be most susceptible to the effect. A value of $UF_H = 3$ (as opposed $UF_H = 10$) was applied because this is a portal-of-entry effect of a direct-acting corrosive, and therefore the response by different subpopulations from anatomic or pharmacokinetic/pharmacodynamic variability is unlikely to differ (NRC, 2001).
- A subchronic-to-chronic uncertainty factor, UF_S, of 3 was applied. While data were not from chronic lifetime exposures, the nasal effects were observed to have a short onset time (Gibb et al. (2000a) estimated a median onset time of 22 days for ulcerated nasal septum, and 172 days for perforated nasal septum). Studies were generally consistent in showing that these effects occur after 1–6 months of exposure. This may indicate that nasal effects occur following short-term occupational exposures to high concentrations of Cr(VI), when significant impaction of large particulates or mists containing Cr(VI) occurs along the nasal passages. As noted in U.S. EPA (2020), if a POD is based on subchronic evidence, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of up to 10 is applied when using subchronic studies to make inferences about lifetime exposure. However, a factor other than 10 may be used depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, 1998a, 1996b, 1994, 1991). Based on the available evidence, it is considered less likely that exposure to Cr(VI) outside of occupational settings (where particulates are larger) would induce nasal perforations/ulcerations at much lower concentrations and smaller particle sizes. (Note: the high response levels at the lowest concentration groups were already accounted for in the LOAEL-to-NOAEL UF selection; the rate of the effect at short onset time shows that there cannot be 10x higher incidence due to prolonged exposure). As a result, a factor of $UF_S < 10$ was applied. Because it is possible that prolonged

exposures to high concentrations may increase the severity of existing nasal lesions after they occur, a value of $UF_S = 3$ (as opposed to $UF_S = 1$) was applied.

Because of the non-uniform distribution of particulates in the lung, extracellular reduction of Cr(VI) by epithelial lining fluid and BAL fluid was not modeled. Inhaled particles may accumulate in susceptible areas such as airway bifurcation sites (Balashazy et al., 2003; Schlesinger and Lippmann, 1978). Localized dosimetry of inhaled particulates in susceptible regions could be significantly higher than the average regional dosimetry estimated by MPPD (ARA (2009)). This assessment assumes that the capacity to reduce Cr(VI) extracellularly in the lung fluid is exceeded in both rodents and humans at all concentrations. Thus, uncertainty factor selections for potential interspecies (UF_A) or intraspecies (UF_H) differences were not influenced by consideration of differences in extracellular lung reduction at concentrations lower than those examined in the available studies.

Table 4-11 is a continuation of Tables 4-9 and 4-10 and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in the tables below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative inhalation reference value for a specific hazard and subsequent overall RfC for Cr(VI).

Table 4-11. Effects in the lower respiratory tract and corresponding derivation of candidate values for Cr(VI)

Endpoint	POD_{HEC} ($\mu g/m^3$)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value ($\mu g/m^3$)
Data for lower respiratory tract effects in male Wistar rats by Glaser et al. (1990)									
Histopathology: histiocytosis	133	LOAEL	3	10	3	3	3	1000	0.13
Histopathology: bronchioalveolar hyperplasia	41.3	$BMDL_{1SD}$	3	10	1	3	3	300	0.14
Cell responses: LDH in BALF	133	LOAEL	3	10	3	3	3	1000	0.13
Cell responses: Albumin in BALF	170	LOAEL	3	10	3	3	3	1000	0.17
Cell responses: Total protein in BALF	133	LOAEL	3	10	3	3	3	1000	0.13
Data for effects in the nasal cavity in humans									
Ulceration of the nasal septum (median) (Gibb et al., 2000a)	3.4	LOAEL	1	3	10	3	3	300	1.1×10^{-2}

Endpoint	POD_{HEC} ($\mu g/m^3$)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value ($\mu g/m^3$)
Nasal mucosal pathology (Cohen et al., 1974)	0.95	LOAEL	1	3	10	3	3	300	3.2×10^{-3}
Ulceration of the nasal septum (Lindberg and Hedenstierna, 1983)	3.3	LOAEL	1	3	10	3	3	300	1.1×10^{-2}

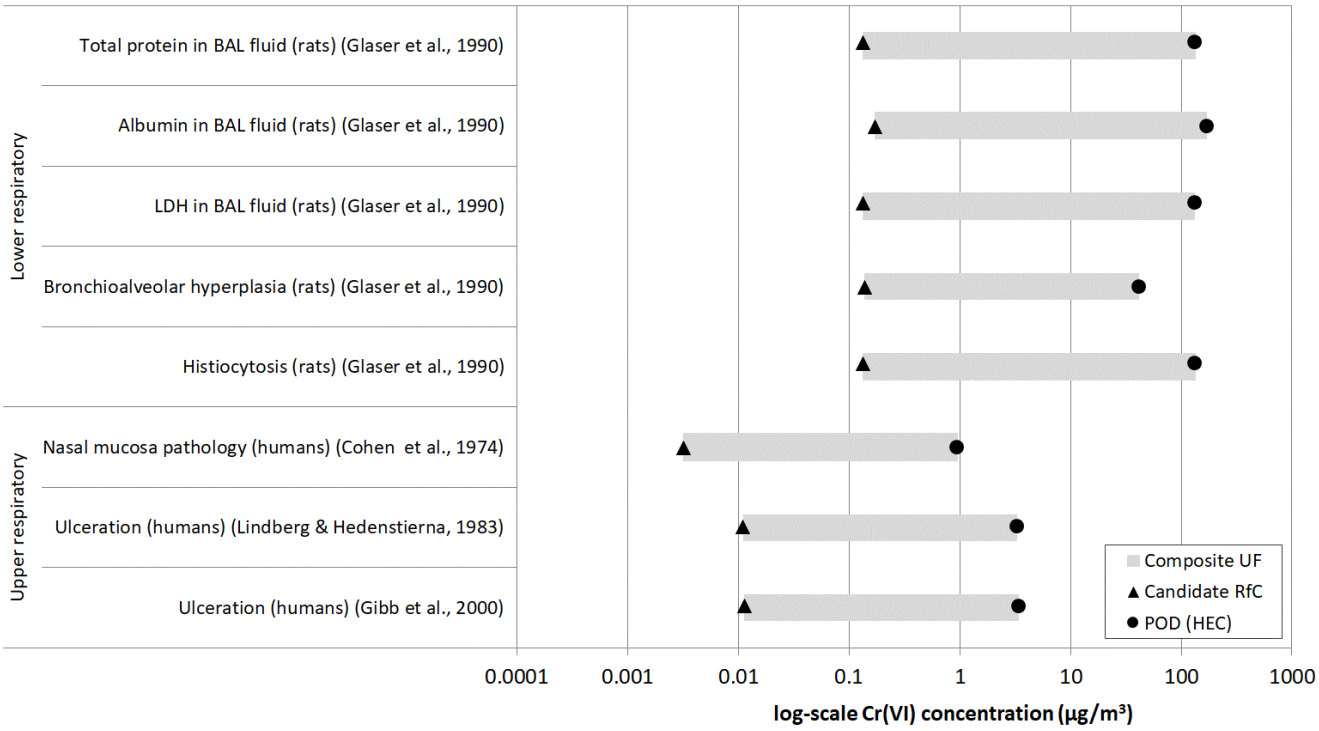

Figure 4-7. Candidate values with corresponding POD and composite UF.

4.2.4. Derivation of Organ/System-Specific Reference Concentrations

Selection of organ-specific toxicity values can be based on the most sensitive outcome, a clustering of values, or a combination. Each candidate value was evaluated with respect to multiple considerations, including strength of evidence, basis of the POD (i.e., BMD vs. NOAEL vs. LOAEL), and dose-response model uncertainties. A confidence level of high, medium, or low was assigned to each organ-specific RfC based on the study(ies) used to derive the candidate value, and the reliability of the associated POD and candidate RfC calculation(s). Confidence in the POD and candidate RfC calculation(s) included considerations of the quality and variability of the exposure assessment in an epidemiology study or the exposure protocols in an animal study. Moreover, higher confidence was placed in the organ-specific RfC when the POD was identified close to the

range of the observed data and the magnitude of exposure was relevant to those experienced in the general U.S. population.

4.2.4.1. *Lower respiratory toxicity*

Cr(VI)-induced cytotoxicity has been observed in epithelial tissues following both inhalation and oral exposures (see Sections 3.2.1 and 3.2.2). Inhaled Cr(VI) in particles, dust, or mists of respirable size may be absorbed into epithelial cells in the lung and lung airways. The organ-specific RfC for lower respiratory tract effects was derived from data in Glaser et al. (1990). Endpoints included lung cellular responses (LDH, albumin, and total protein in BAL fluid), and changes in lung histopathology (histiocytosis and bronchioalveolar hyperplasia). Because most of these endpoints had the same LOAEL and uncertainty factors, they produced essentially the same candidate RfCs (note: albumin in BAL fluid differed from the others slightly due to selection of a different RDDR extrapolation region for animal-to-human extrapolation). BMD modeling was performed on data of bronchioalveolar hyperplasia, and the resulting candidate RfC (which used a lower uncertainty factor) supported the NOAEL-derived cRfCs. The osRfC for lower respiratory system effects was taken as the value of the candidate RfCs for cellular responses (total protein and LDH in BAL fluid) and histopathology findings (histiocytosis and bronchioalveolar hyperplasia) resulting in an osRfC of 0.13 $\mu g/m^3$ (rounded to 0.1 $\mu g/m^3$, or 1×10^{-4} mg/m^3).

The relatively small number of *medium* confidence studies evaluating noncancer lower respiratory effects decreases the confidence of this organ-specific RfC. In addition, the endpoint was derived from subchronic rodent data. Human data for noncancer lower respiratory tract effects of Cr(VI) are scarce because studies published prior to the availability of standardized spirometry guidelines from the American Thoracic Society (first developed in 1979) (ATS/ERS, 2019) were considered uninformative for pulmonary function. A factor that increased confidence was the clear dose-response observed for multiple lower respiratory endpoints in rodents.

4.2.4.2. *Upper respiratory toxicity*

As noted earlier, Cr(VI) is cytotoxic and there is high confidence that Cr(VI) induces effects at the portals of entry. Furthermore, effects in the nasal cavity of humans are well documented by occupational studies (OSHA, 2006). The osRfC for effects in the upper respiratory tract were based on ulcerated nasal septum observed by the Gibb et al. (2000a) occupational study. While the study reported multiple other nasal endpoints (irritated, perforated, and bleeding nasal septum), ulcerated nasal septum was chosen because of its severity and high incidence (63% of the cohort having the clinical finding). Gibb et al. (2000a) had higher sample sizes and better exposure data than the alternative studies by Cohen et al. (1974), and Lindberg and Hedenstierna (1983).

The Baltimore plant studied by Gibb et al. (2000a) had a rigorous personal and air monitoring system that spanned a period of decades (see Table 4-23 in Section 4.4.5). This greatly increased confidence in the reported air concentrations and worker exposures. While the Lindberg and Hedenstierna (1983) used both area and personal air samplers, the recorded data only

spanned 13 days. Furthermore, the defined concentration ranges (<2–20 μg Cr(VI)/m^3) by Lindberg and Hedenstierna (1983) only constituted average workday concentrations (peak values were noted to be higher, but only limited concentration data are presented). Characterization of the nasal endpoints by Cohen et al. (1974) were highly detailed, and the study employed only air measurements consistent with current NIOSH recommendations (Andrews and O'Connor, 2020; NIOSH, 2013a). However, the sample size was small, and the breathing-zone air samples represented only a snapshot in time (and not the long-term exposure of the workers over time). The osRfC for upper respiratory tract effects is based on the LOAEL for ulcerated nasal septum in humans reported by Gibb et al. (2000a), resulting in an osRfC of 1.1 × 10^{-2} μg/m^3 (rounded to 1 × 10^{-5} mg/m^3). Because only LOAELs could be obtained from the datasets, and because the estimated effect incidences were high at the LOAEL (63%), there is uncertainty in the dose-response relationship at lower concentrations. For the Gibb et al. (2000a) study, effects in the nasal cavity were observed after a few months of exposure (median time on the job of 86–418 days), and it is unknown how the effect severity may increase over a lifetime of exposure. These factors decrease confidence in the organ-specific RfC for upper respiratory tract effects. Additional uncertainties relevant to upper respiratory tract effects are described in detail in Section 4.2.6. Factors that increase confidence in the organ-specific RfC for upper respiratory tract effects include the consistency at which this effect was observed (generally between 2–20 μg Cr(VI)/m^3 with early onset time), and the thorough air sampling programs implemented for the Baltimore Cohort (see Table 4-23) (Gibb et al., 2000a).

Table 4-12. Organ/system-specific reference concentrations (RfCs) and overall RfC for Cr(VI)

Effect	Basis	osRfC mg/m^3	Exposure description	Confidence
Lower respiratory	Increase in total protein and LDH in BAL fluid, and histiocytosis and bronchioalveolar hyperplasia in male rats Glaser et al. (1990)	1 × 10^{-4}	90-day rat study	Medium
Upper respiratory	Ulcerated nasal septum of humans Gibb et al. (2000a)	1 × 10^{-5}	Occupational exposure	Medium
Overall RfC	Ulcerated nasal septum	1 × 10^{-5}	Occupational exposure	Medium

As noted in Section 4.2.8, the prior assessment developed separate RfCs for "chromic acid mists and dissolved hexavalent chromium aerosols," and for "hexavalent chromium dusts." The RfC for chromic acid mists was based on human occupational exposure to chromic acid (H_2CrO_4) at a chrome-plating facility by Lindberg and Hedenstierna (1983), while the RfC for dusts was based on

data for rodent exposure to sodium dichromate ($Na_2Cr_2O_7$) aerosols by Glaser et al. (1990; 1985). The current database now includes noncancer data from the Baltimore chromate production plant (Gibb et al., 2000a), which studied effects in humans occupationally exposed to a variety of chromium species in dust form, including sodium chromates (Na_2CrO_4) and dichromates ($Na_2Cr_2O_7$) (Hayes et al., 1979). As shown in Table 4-11 the RfCs for human nasal effects are the same between the Gibb et al. (2000a) study (which contained chromium dusts) and Lindberg and Hedenstierna (1983) (primarily chromic acid mists). Lindberg and Hedenstierna (1983) observed that ulceration of the nasal septum occurred only in the highest peak exposure group (20−48 μg Cr(VI)/m^3) and the highest daily exposure group (>2−20 μg Cr(VI)/m^3). This is supportive of Gibb et al. (2000a), which reported ulceration of the nasal septum at a median concentration of approximately 10 μg Cr(VI)/m^3. Therefore, the RfC for upper respiratory tract effects is applicable to both forms of Cr(VI) (mists and dusts). EPA also considers the RfC for lower respiratory tract effects applicable to both forms of Cr(VI).

The previous distinction in RfCs drawn between mists and dusts is no longer supported. However, distinctions are presented via the organ-specific RfCs (upper vs. lower respiratory tracts), and these may be a direct function of particle or droplet size. It is generally known that large inhaled particles (with diameter >5 μm) will deposit in the extrathoracic region, particles greater than 2.5 μm are generally deposited in the tracheobronchial regions, and particles less than 2.5 μm are generally deposited in the pulmonary region (OSHA, 2006). The rodent study of $Na_2Cr_2O_7$ aerosols by Glaser et al. (1990; 1985) likely induced effects in the lower respiratory tract due to the small particle sizes achieved by the experiment (MMAD < 0.4 μm). For the human occupational studies, particle and droplet sizes may have been larger, causing a larger proportion of Cr(VI) to impact in the nasal airways.

4.2.5. Selection of the Overall Reference Concentration

An overall RfC of 1×10^{-5} mg/m^3 was selected. The overall RfC was based on effects in the upper respiratory tract (ulceration of the nasal septum), because of the two endpoints representative of respiratory tract effects it is the more sensitive effect and will be protective of noncancer lower respiratory tract effects and systemic effects. Additional considerations of uncertainty associated with this RfC are noted here and below in section 4.2.6. It was derived using a LOAEL, where the incidence of the effect was high and the time of onset relatively short. The occupational cohort (Gibb et al., 2000a) consisted of a population of mostly adult males and may not have included sensitive individuals. It is uncertain if or how the endpoint severity may be affected by lifetime chronic exposures.

4.2.6. Uncertainties in the Derivation of Reference Concentration

4.2.6.1. *Onset time for nasal effects*

The time between first exposure and development of nasal effects varies depending on the severity of the effect, but nasal effects generally occur within 1 year of initial exposure for more severe effects, and 1–3 months for less severe effects. Gibb et al. (2000a), the only prospective study of the development of nasal effects reported the time to event in days (mean [median]) for irritation (89 [20]), ulceration (86 [22]), perforation of the septum (313 [172]), and bleeding nasal septum (418 [92]) (Gibb et al., 2000a). Cross-sectional studies reported a similar time to event periods based on self-reported interview data (Lindberg and Hedenstierna, 1983; Cohen et al., 1974). Cohen et al. (1974) reported that severity of pathology increased with longer exposure times and prevalence of ulceration or perforation in the study population was higher at 94% in workers who had worked at the plant for more than 1 year at the time data were collected compared to 57% among workers who had worked for less than a year at the same plant. More recently, Singhal et al. (2015) showed that severity of nasal outcomes increased with years of exposure in both chromate manufacturing and chrome electroplating workers. The early onset-time, combined with the fact that incidences were high at the lowest concentration (the lowest concentration in this occupational setting is still high relative to environmental levels) leads to uncertainty in the extrapolation from occupational exposure to continuous lifetime exposure.

4.2.6.2. *Hand-to-nose transfer*

Only one of the candidate RfC studies reported hand-to-nose transfer of Cr(VI) originating from surface touching (Cohen et al., 1974). Surface contamination of Cr(VI) throughout workplace environments (including on gloves and other personal protective equipment), and detection of Cr(VI) on the hands of employees have been documented (Ceballos et al., 2017; Lucas and Kramkowski, 1975; Cohen and Kramkowski, 1973). However, no quantitative data were available to adjust for this potential route of exposure.

4.2.6.3. *Susceptible populations*

Quantitative analysis of effects in the lower respiratory tract were based on animal data, while analysis of effects in the upper respiratory tract were based on occupational studies of adult humans. Data for these effects were not available in susceptible populations, such as children or those with preexisting respiratory conditions.

4.2.7. Confidence Statement

An overall confidence level of **High**, **Medium**, or **Low** was assigned to reflect the level of confidence in the study(ies) and hazard(s) used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

The confidence in the overall chronic RfC is **medium**. The RfC for upper respiratory tract effects is based on the LOAEL for ulcerated nasal septum in humans reported by Gibb et al. (2000a), resulting in an RfC of 1×10^{-5} mg/m^3. While there is high confidence that inhaled Cr(VI) can induce effects in the nasal cavity of humans, quantitative characterization of these endpoints have uncertainties. The available studies did not have enough exposure groups or individual-level data adequate for a dose-response analysis, and only LOAELs could be obtained from the datasets. For the Gibb et al. (2000a) study, effects in the nasal cavity were observed after a few months of exposure (median time on the job of 86–418 days), and it is unknown how the effect severity may increase over a lifetime of exposure. Because the estimated effect incidences were high at the LOAEL (63%), there is uncertainty in the dose-response relationship at lower concentrations. As a result, the confidence in the RfC for upper respiratory effects is medium.

4.2.8. Previous IRIS Assessment: Inhalation Reference Concentration

The previous IRIS assessment contained two RfCs for Cr(VI). An RfC for "chromic acid mists and dissolved hexavalent chromium aerosols" and an RfC for "hexavalent chromium dusts" were posted on the IRIS database in 1998. As noted in Section 4.2.4, health effects induced by inhalation exposure to Cr(VI) are expected to differ due to particle size distribution. These differences are now reflected in the derivation of organ-specific RfCs, which are strongly dependent on particle sizes rather than other chemical properties. Larger particles are more likely to affect the nasal airways, while smaller particles can affect the lower airways.

The 1998 RfC for Cr(VI) acid mists and dissolved aerosols was based on the human study by Lindberg and Hedenstierna (1983). A LOAEL for nasal septum atrophy of 2 μg/m^3 was identified based on the lower bound of the 2-20 μg/m^3 range, and this value was adjusted using a continuous exposure adjustment factor, and an adjustment factor for occupational and 24-hour average breathing rates. This resulted in a LOAEL for continuous exposure of 0.714 μg/m^3. A total uncertainty factor of 90 was applied: 3-fold for extrapolation from a subchronic to a chronic exposure, 3-fold for extrapolation from a LOAEL to a NOAEL, and 10-fold for interhuman variation. This resulted in an RfC of 0.008 μg/m^3 (8×10^{-6} mg/m^3) for hexavalent chromic acid mists and dissolved hexavalent chromium aerosols. The current assessment derived a different LOAEL for the Lindberg and Hedenstierna (1983) study, because most cases (7/8) of nasal ulceration in the 2-20 μg/m^3 group had peak exposure levels at or above 20 μg/m^3.

The previous RfC for Cr(VI) dusts was based on the studies by Glaser et al. (1990; 1985) and used the modeling and data analysis of this dataset published by Malsch et al. (1994). Malsch et al. (1994) developed BMCs for lung weight, lactate dehydrogenase (LDH) in BAL fluid, protein in BAL fluid, albumin in BAL fluid, and spleen weight. The Malsch et al. (1994) analysis defined the benchmark concentration as the 95% lower confidence limit on the dose corresponding to a 10% relative change in the endpoint compared to the control. A continuous exposure adjustment factor was applied, and the maximum likelihood model was used to fit continuous data to a polynomial

mean response regression, yielding maximum likelihood estimates of 36–78 μg/m^3 and BMCs of 16–67 μg/m^3. LDH was the most sensitive endpoint (BMC of 16 μg/m^3) and was the basis of the 1998 IRIS assessment RfC for Cr(VI) dusts. An RDDR of 2.1576, derived by methods outlined in U.S. EPA (1994), was applied to this value to extrapolate a human equivalent concentration. A total uncertainty factor of 300 was applied: 10-fold for the less-than-lifetime exposure, 10-fold for variation in the human population, and 3-fold to account for pharmacodynamic differences not accounted for by the RDDR. This resulted in an RfC of 1×10^{-4} mg/m^3 for hexavalent chromium dusts, which is the same as the value derived in this assessment for lower respiratory tract effects (using the same study and similar methods).

4.3. ORAL SLOPE FACTOR FOR CANCER

The oral slope factor (OSF) is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. The OSF can be multiplied by an estimate of lifetime exposure (in mg/kg-day) to estimate the lifetime cancer risk.

4.3.1. Analysis of Carcinogenicity Data

The animal database for cancer consisted of a chronic 2-year drinking water bioassay which found "clear evidence of carcinogenic activity" of Cr(VI) in male and female rats and mice (NTP, 2008). These results were based on increased incidences of squamous cell neoplasms in the oral cavity of rats, and increased incidences of neoplasms in the small intestine of mice. The data from NTP (2008) indicate a dose-response relationship in both species.

Human dose-response data for cancer via the oral route were not suitable for dose-response analysis. The lack of individual estimates of exposure, the uncertain nature of the mortality data, and the potential impact of confounding made it difficult to draw conclusions (see Section 3.2.3). Human cancer data via the inhalation route of exposure (for either lung tumors described in Section 4.4, or GI tract risk described in the meta-analysis in Section 3.2.2) were not used for oral slope factor derivation because route-to-route extrapolations were not considered in this assessment (see Protocol, Appendix A).

4.3.2. Dose-Response Analysis—Adjustments and Extrapolations Methods

A benchmark dose (BMD) approach was used to model the dose-response data. This method is described in detail in Section 4.1.2. Because a mutagenic mode-of-action for Cr(VI) carcinogenicity via the oral route of exposure (see Section 3.2.3) is "sufficiently supported in (laboratory) animals" and "relevant to humans," EPA used a linear low dose extrapolation from the POD in accordance with *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The multistage model was selected for dose-response analysis because it is consistent with low dose linearity, it is sufficiently flexible for most cancer bioassay data, and its use provides consistency across cancer dose-response analyses (Gehlhaus et al., 2011).

For tumors of the small intestine of mice, a PBPK model was used to extrapolate the rodent dose-response model results to a human equivalent dose, using the same methodology applied for noncancer effects (Section 4.1.2). The internal dose used for mouse-to-human extrapolation was the $BW^{3/4}$-adjusted Cr(VI) dose that is estimated to escape gastric reduction. The mean result from Monte Carlo analysis was used as the POD for the OSF, as opposed to the lower 1% value (which was used for the POD of the RfD). This is because intraspecies variability in pharmacokinetics and pharmacodynamics is not incorporated into cancer risk assessment (U.S. EPA, 2006c), with the exception for early-life considerations noted in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). Uncertainty factors are not applied during rodent-to-human extrapolation of cancer dose-response data. For comparative purposes, the $BW^{3/4}$ scaling approach without Cr(VI) gastric reduction modeling or Monte Carlo analysis is also presented. This can be interpreted as the result for a susceptible subpopulation having high gastric pH (>4.0) and Cr(VI) gastric juice reduction capacity equivalent to rodents (see Appendix C.1.5).

For tumors in the oral cavity of rats, there is uncertainty regarding the appropriate internal dose metric. Mice did not exhibit tumors of the oral cavity, but in a separate bioassay were observed to have higher oral tissue chromium levels than rats following 90-day drinking water exposure (Kirman et al., 2012). Mice rarely exhibit oral tumors from NTP bioassays, even for chemicals inducing oral tumors in rats (Ibrahim et al., 2021; NTP, 2008)[68]. Thus, mice may be less susceptible to tumors of the oral cavity due to factors that cannot be accounted for using PBPK modeling. There were no observed nonneoplastic lesions in the oral mucosa of rats or mice following either the chronic or subchronic high dose NTP Cr(VI) drinking water bioassays (Witt et al., 2013). Unlike for the mouse, where tumors were observed in GI organs posterior to the stomach (where most Cr(VI) reduction occurs), tumors of the rat oral cavity occur in tissues where Cr(VI) exposure is not mitigated by extracellular reduction in the stomach. As a result, species differences in Cr(VI) reduction in the stomach are not relevant for the dose-response analysis of rat oral tumors. Site-specific PBPK models of Cr(VI) kinetics in the oral cavity epithelium are not available. In the absence of an adequately developed theory or information to develop and characterize an oral portal-of-entry dosimetric adjustment factor, application of $BW^{3/4}$ scaling is recommended (U.S. EPA, 2011c, 2005a).

[68]Of the 24 test articles associated with site-specific neoplasia that produced positive, clear or some evidence of carcinogenicity in the oral cavity (NTP, 2020), only one (1,2,3-trichloropropane) induced tumors of the oral cavity in mice. All other test articles induced tumors in the oral cavity of male or female rats. With the exception of Cr(VI), three chemicals were found to induce both oral and small intestinal tumors (2,2-bis(Bromomethyl)-1,3-propanediol, C.I. Direct blue 15, C.I. Acid red 114), although they only induced these effects in rats. In general, tumors of the small intestine are more rare in rats (compared to mice), and tumors of the oral cavity are more rare in mice (compared to rats) (see Appendix D.5).

4.3.3. Derivation of the Oral Slope Factor

The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = $0.1/BMDL_{10}$). This slope, a 95% upper confidence limit represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination. Results for all tumor types are listed in Table 4-13.

Table 4-13. Summary of the oral slope factor derivations

Species/ sex	Model	BMR	BMD mg/kg-d[a]	BMDL mg/kg-d[a]	Extrapolation Method	Internal rodent dose mg/kg-d[b]	Internal dose POD mg/kg-d[c]	POD_{HED} mg/kg-d[d]	OSF Per mg/kg-d
Adenomas or Carcinomas in the mouse small intestine (NTP, 2008)									
Mice (M)	1° MS	10	1.44	1.05	PK	0.173	0.0274	0.319	0.313
					$BW^{3/4}$	N/A	N/A	0.166	0.602
Mice (F)	1° MS	10	1.34	1.03	PK	0.169	0.0267	0.316	0.317
					$BW^{3/4}$	N/A	N/A	0.163	0.613
Squamous cell carcinoma or squamous cell papilloma in oral mucosa or tongue (NTP, 2008)									
Rats (M)	1° MS	10	6.01	3.35	$BW^{3/4}$	N/A	N/A	0.917	0.109
Rats (F)	1° MS	10	4.25	2.70	$BW^{3/4}$	N/A	N/A	0.645	0.155

[a]Units of administered mg/kg-d Cr(VI) dose.
[b]Dose escaping stomach reduction in rodent (mg/kg-d) estimated by PK modeling.
[c]BW3/4 scaling adjustment of the internal rodent dose (dose escaping reduction multiplied by (BWA/BWH)1/4 , where BWH = 80 kg and BWA is set to study-specific time-weighted average (TWA) values (these same study-specific BW values were also used in the PK modeling). TWA BWA = 0.450 kg for male rats, and TWA BWA = 0.260 kg for female rats at the 2-year time period in NTP (2008). TWA BWA = 0.05 kg for male and female mice at the 2-year time period in NTP (2008).
[d]PODHED in units of mg/kg-d Cr(VI) oral dose ingested by humans. For the PK method, this is the mean value of 20000 Monte Carlo PK simulations needed to achieve the internal dose POD (see Appendix C.1.5 for details). For the standard $BW^{3/4}$ method, no additional adjustments beyond $BW^{3/4}$ scaling of the rodent dose are applied.

The OSF for Cr(VI) was chosen to be the value derived from small intestine tumors in male and female mice using PBPK modeling, 0.3 $(mg/kg\text{-}d)^{-1}$.

4.3.4. Application of age-dependent adjustment factors

Because a mutagenic mode of action for hexavalent chromium carcinogenicity is sufficiently supported in laboratory animals and is relevant to humans (see Section 3.2.3), and in the absence of chemical-specific data to evaluate differences in age-specific susceptibility, increased early-life susceptibility to hexavalent chromium is assumed and ADAFs should be applied, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The oral slope factor of 0.3 $(mg/kg\text{-}day)^{-1}$, calculated from data applicable to adult exposures, does not reflect presumed early-life susceptibility to this chemical.

Example calculations for estimating cancer risks based on age at exposure are provided in Section 6 of the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

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

To illustrate the use of the ADAFs established in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), OSF calculations are presented for three exposure duration scenarios, including full lifetime. For oral exposures assuming Cr(VI) exposure-response equivalence across age groups (i.e., equivalent risk from equivalent exposure levels, independent of body size), the ADAF calculation is fairly straightforward. The partial and lifetime risks (per mg/kg-d) are presented below in Table 4-14.

Table 4-14. Application of ADAFs for 70-year exposure to Cr(VI) from ages 0 to 70

Age group	ADAF	Slope factor (per mg/kg-d)	Duration adjustment	Partial risk (per mg/kg-d)
0–<2 yrs	10	0.3	2 yrs/70 yrs	0.0857
2–<16 yrs	3	0.3	14 yrs/70 yrs	0.180
≥16 yrs	1	0.3	54 yrs/70 yrs	0.231
			Total risk	0.497

Note that the partial risk for each age group is the product of the values in columns 2–5 (e.g., 10 × 0.3 × 2/70 = 0.0857 for exposures from age 0 to <2 years), and the total risk is the sum of the partial risks. Thus, a lifetime estimate for the OSF for exposure starting at birth is 0.5 (per mg/kg-d).

If calculating the cancer risk for a 30-year exposure to a constant average daily dose of 0.0001 mg Cr(VI)/kg-day from ages 0 to 30 years, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks would be ($10 \times 0.3 \times 0.0001 \times 2/70 = 8.6 \times 10^{-6}$), ($3 \times 0.3 \times 0.0001 \times 14/70 = 1.8 \times 10^{-5}$.), and ($1 \times 0.3 \times 0.0001 \times 14/70 = 6 \times 10^{-6}$), resulting in a total risk estimate of 3.3×10^{-5}.

If calculating the cancer risk for a 30-year exposure to a constant average daily dose of 0.0001 mg Cr(VI)/kg-day from ages 20 to 50 years, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks would be 0, 0, and ($1 \times 0.3 \times 0.0001 \times 30/70 = 1.3 \times 10^{-5}$), resulting in a total risk estimate of 1.3×10^{-5}.

4.3.5. Uncertainties in the Derivation of the Oral Slope Factor

Because the studies and pharmacokinetics methods used to derive the OSF are the same as those used to derive the RfD, the major uncertainties related to OSF derivation are outlined in Section 3.3 and Section 4.1.6. Additional information on susceptible populations is provided in Section 3.3.1. Briefly,

- Uncertainties persists in the PBPK models of the human and mouse stomach. Population variability in kinetic parameters is unknown, and it is likely that gastric contents and microbiota contribute to interindividual variation.
- Uncertainty in the choice of the tumor type and internal dose metric for cross-species extrapolation.
- Cr(VI) detoxification in the stomach for populations with elevated stomach pH (consumers of medicine to treat acid reflux, hypochlorhydria individuals) may differ from standard health individuals.
- There may be higher susceptibility for carriers of mutated cystic fibrosis transmembrane conductance regulator (CFTR) gene (see Sections 3.2.3.4 and 3.3.1).

Individuals taking medication to treat gastroesophageal reflux disease (GERD), including calcium carbonate-based acid reducers and proton pump inhibitors, have an elevated stomach pH during treatment. This is known to be a significant fraction of the population since up to 20% of the population is afflicted by GERD, and the gastric pH for these individuals may be above 4 throughout the day during successful treatment (Delshad et al., 2020; GBD 2017, 2020; Lin and Triadafilopoulos, 2015; Burdsall et al., 2013; Atanassoff et al., 1995). A sensitivity analysis was performed on the human model (Appendix C.1.5), assuming a baseline stomach pH = 4 (as opposed to 1.3). It was found that for internal doses near those of the cancer PODs for mice, the mean human equivalent dose for a population with baseline gastric pH = 4 would be approximately ½ that of the standard population with baseline pH = 1.3. As a result, the OSF for this population would be 2x more stringent. Similarly, the OSF estimated by default approaches ($BW^{3/4}$ scaling and no adjustment for gastric reduction) would be health-protective for this population, since that method implicitly assumes that humans and rodents have the same gastric pH (>4) and reduction capacity. After rounding, the adult-based OSF for $BW^{3/4}$ scaling (0.6 per mg/kg-d) is exactly 2x the adult-based OSF estimated by PBPK modeling (0.3 per mg/kg-d). Under the $BW^{3/4}$ scaling assumption, the lifetime ADAF-adjusted value would also be exactly 2x more stringent (1.2 per mg/kg-d).

Table 4-15 provides an overview summarizing the uncertainties and their impact on the OSF.

Table 4-15. Summary of uncertainties in the derivation of oral slope factor values for Cr(VI)

Consideration	Impact on unit risk	Decision	Justification
Target organ	↓ OSF, 3-fold, if oral tumors selected	Small intestine tumors (adenomas or carcinomas of the duodenum or jejunum or ileum in mice)	Tumor site is concordant across rats and mice in the GI tract as a whole (small intestine and mouth), increasing support for its relevance to humans. As there are no data to support any one result as most relevant for extrapolating to humans, the most sensitive result for GI tract tumors was used to derive the oral slope factor.
Data set	None	NTP (2008)	NTP (2008) was a high confidence study and the only to evaluate potential carcinogenicity in multiple organs and multiple species following chronic drinking water exposure.
Cross-species scaling dose metric	Alternatives could ↓ or ↑ slope factor	mg/kg-d Cr(VI) emptied from stomach, adjusted by $BW^{3/4}$ scaling	The amount of Cr(VI) available for absorption into the small intestine is a function of how much Cr(VI) will escape the stomach unreduced. Applying the pyloric flux dose metric defined in Thompson et al. (2014) (daily mg Cr(VI) emptied from stomach, per L small intestine) would slightly decrease the OSF ($BW^{3/4}$ scaling is similar as scaling by small intestine volume). Applying $BW^{3/4}$ scaling without taking into account interspecies differences in gastric reduction would increase the OSF by 2x.
Low dose extrapolation	↓ cancer risk estimate would be expected with the application of nonlinear low dose extrapolation	Linear extrapolation from POD (based on mutagenic MOA)	Available MOA data support linearity (mutagenicity is a primary MOA of Cr(VI)). See Appendix D.3 for an uncertainty analysis of the low dose extrapolation method
Statistical uncertainty at POD	↓ OSF 1.4-fold if BMD used as the POD rather than BMDL	BMDL (preferred approach for calculating plausible upper-bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure at 10% extra risk of alimentary tract tumors.
Dose-response modeling	Alternatives could ↓ or ↑ slope factor	Multistage-model	No biologically based models for Cr(VI) were available. Multistage models are sufficiently flexible for most cancer bioassay data, and their use provides consistency across cancer dose-response analyses. See Appendix Section C.1.5 for additional details on the impact of alternative dose metrics.
Sensitive subpopulations	↑ OSF to unknown extent	ADAFs are recommended for early-life exposures	No chemical-specific data are available to determine the range of human pharmacodynamic variability or sensitivity. Deriving an OSF from populations with high baseline gastric pH would lead to a significantly higher OSF (over 2x higher).

4.3.6. Previous IRIS Assessment: Oral Slope Factor

The previous IRIS assessment for hexavalent chromium was posted to the IRIS database in 1998. In that assessment, EPA concluded that the oral carcinogenicity of hexavalent chromium could not be determined (and was thus classified as Group D under the 1986 classification guidelines). At the time, only one study in humans suggested an association with stomach cancer, but other human and animal studies did not report similar effects. Therefore, no oral slope factor was derived.

4.4. INHALATION UNIT RISK FOR CANCER

The inhalation unit risk (IUR) is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed. The IUR can be multiplied by an estimate of lifetime exposure (in $\mu g/m^3$) to estimate cancer risks over a lifetime or partial lifetime.

In 1998, the EPA IRIS Toxicological Review of Hexavalent Chromium classified Cr(VI) as a "known human carcinogen by the inhalation route of exposure" based on consistent evidence that inhaled Cr(VI) causes lung cancer in humans and supporting evidence of carcinogenicity in animals. The same conclusion has since been reached by other authoritative federal and state health agencies and international organizations and the carcinogenicity of Cr(VI) is considered to be well-established for inhalation exposures (TCEQ, 2014; IPCS, 2013; NIOSH, 2013b; IARC, 2012; CalEPA, 2011; NTP, 2011; OSHA, 2006).

4.4.1. Analysis of Carcinogenicity Data

This section focuses on identifying additional appropriate studies to update the quantitative exposure-response analysis and the derivation of the IUR. More recent epidemiologic studies have been identified in the peer-reviewed literature which include higher quality exposure data, longer follow-up times, larger sample sizes, and more sophisticated analyses than were available in 1998. While the focus of the updated cancer analysis was evaluation of new information and other studies that were not evaluated in the 1998 IRIS assessment, EPA did not exclude studies published prior to 1998. Having judged the evidence of hazard for carcinogenicity of inhaled Cr(VI) to be well-established, EPA focused on studies that could inform estimation of the exposure-response function which could be used to derive an IUR.

4.4.1.1. *Identification of studies for the derivation of a Cr(VI) inhalation unit risk*

Study selection

A title and abstract screening of human health studies obtained from the literature searches described in Sections 1.2 and 2.1, and backwards searching using reference lists of screened studies, identified 61 human lung cancer references. These studies then underwent full-text screening for exposure-response data that may be informative for derivation of a revised inhalation unit risk. Studies needed to be epidemiological analyses examining quantitative measures of

chromium exposure in relation to lung cancer incidence or mortality risk. Studies were excluded if Cr(VI) measurements in air, or convertible equivalents such as CrO_3, were not presented, or if group-level exposure assignments were based on job title (and not chromium measurements) (see Table D-28 in Appendix D.4). Applying these criteria, there were 22 lung cancer references identified as potentially informative for exposure-response analysis.

All 22 studies were based on occupational cohorts, and many followed the same worksites or worker populations over time. For cohorts with multiple follow-up studies, EPA included only the most recent follow-up, and used the prior studies to obtain information relevant to analysis of data and study quality (see Table D-29 in Appendix D.4). Of the 22 studies, five independent cohort studies evaluating Cr(VI) exposure and the risk of lung cancer were obtained after restricting to the most recent cohort follow-up data (Figure 4-8 and Table 4-16). These were: (1) a chromate facility in Baltimore, MD (Gibb et al., 2020; Gibb et al., 2015; Gibb et al., 2000b); (2) a chromate facility in Painesville, OH (Proctor et al., 2016); (3) two chromate facilities in Germany (Leverkusen and Uerdingen) (Birk et al., 2006); (4) the IARC multicenter cohort of welders in the European Union (Gerin et al., 1993); and (5) two chromate facilities in the United States (Corpus Christi TX and Castle Hayne NC) (Luippold et al., 2005). A sixth study (AEI, 2002) did not include new data, but was a pooled analysis of the four plants evaluated in Birk et al. (2006) and Luippold et al. (2005).

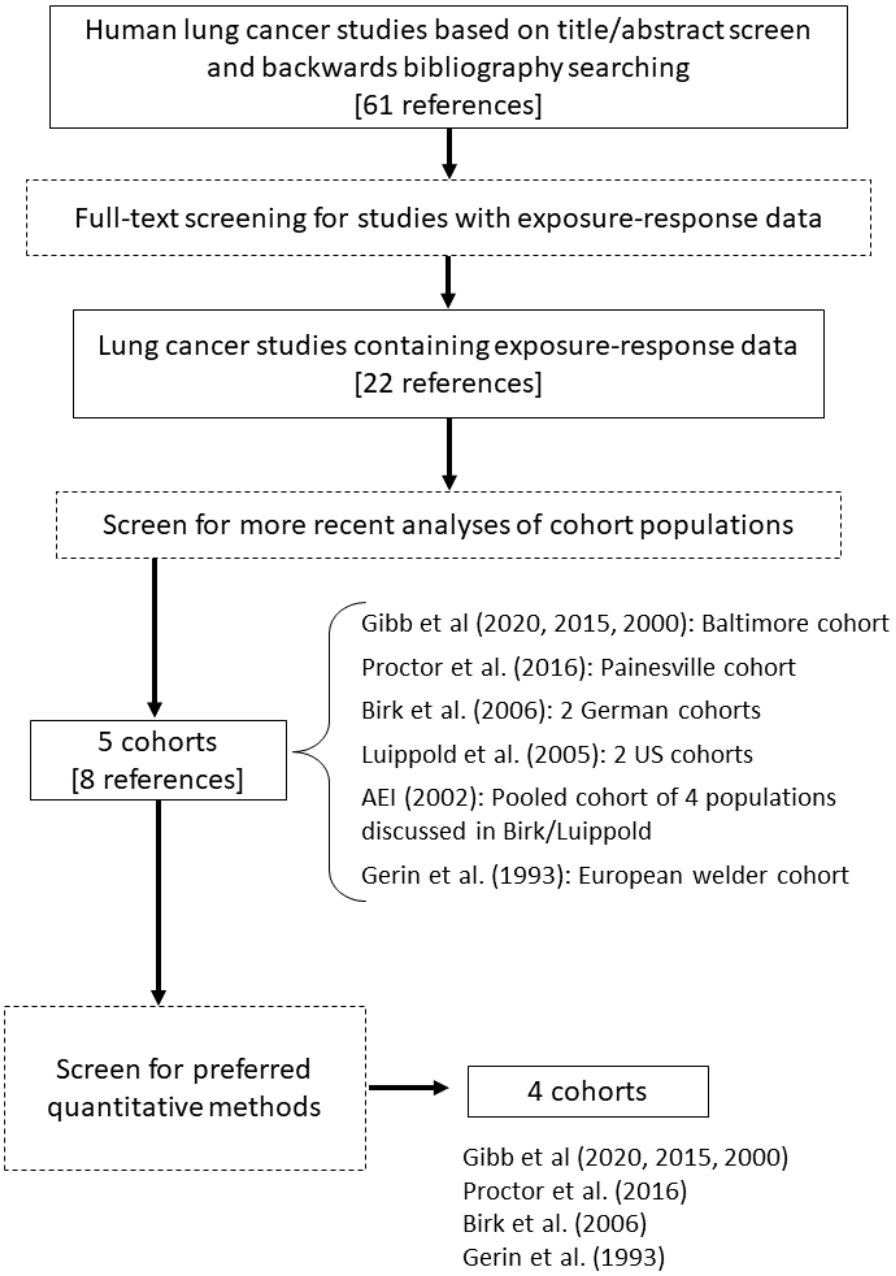

Figure 4-8. Literature screening results for studies containing exposure-response data of Cr(VI) and lung cancer.

The next step was to evaluate the quantitative methods used in each of the analyses. It was preferred that exposure-response analyses were conducted using estimated airborne concentrations of speciated Cr(VI) compounds from which a slope[69] and its standard error could be obtained. Studies were available that presented results from models using a continuous measure of

[69]The beta coefficient describing the function of exposure-response relationship between exposure to Cr(VI) in air, on a continuous scale, and the risk of lung cancer.

exposure, so the four that did not (e.g., studies that only presented an overall SMR) were excluded: Davies et al. (1991), Luippold et al. (2005), AEI (2002), and Girardi et al. (2015). An overview of all studies excluded for exposure-response analysis of lung cancer in humans is provided in Appendix D4 Tables D-28 through D-30. The remaining four studies were then evaluated for risk of bias and sensitivity. Study evaluation included consideration of exposure assessment, outcome ascertainment, population selection, confounding, selective reporting, sensitivity, and data analysis [see Protocol Section 6.2 (Appendix A) for more details]. Considerable focus was placed on factors that could notably affect the magnitude and direction of the effect estimates, including potential for exposure measurement error, confounding, missing data, and the specific statistical analyses conducted. Summaries of the study evaluations are presented in Table 4-16 along with the overall confidence rating. Details of those evaluations are presented in HAWC (click here).

Table 4-16. Summary of included studies considered for the derivation of an inhalation unit risk for Cr(VI) and overall confidence classification. Click to see interactive data graphic for rating rationales.

	Reference	Study description	Study evaluation							
			Exposure	Outcome	Selection	Confounding	Analysis	Sensitivity	Sel. reporting	Overall confidence
Included	Gibb et al., (2020; 2015; 2000b)[a]	Occupational cohort (*n* = 2,354 male workers) in the U.S. exposed 1950–1985 and followed until 2011.	G	G	A	A	G	A	A	High
	Proctor et al. (2016)	Occupational cohort (*n* = 714 male workers) in the U.S. exposed 1940–1972 and followed until 2011.	A	A	G	D	G	A	A	Medium
	Birk et al. (2006)	Occupational cohort (*n* = 901 male workers) in Germany exposed 1958–1998 and followed until 1998.	D	A	A	A	A	D	A	Low
	Gerin et al. (1993)	Pooled IARC multicenter occupational cohorts (*n* = 11,092 male welders) across 135 companies in 9 EU countries exposed during various periods 1946–1986.	D	A	A	D	A	A	A	Low

[a]Three studies were used to represent the Baltimore, MD cohort, as they had essentially the same worker population.

Three studies describing one cohort were classified as *high* confidence: Gibb et al., (2020; 2015; 2000b) (the Baltimore MD cohort); and one was classified as *medium* confidence: Proctor et al. (2016) (the Painesville OH cohort). The remaining studies were *low* confidence. The *high* and *medium* confidence studies were advanced for further consideration in the derivation of the IUR for

Cr(VI). Overviews of the two cohorts and their analyses are provided below followed by an analysis of the preferred characteristics for candidate principal studies for IUR development from occupational cohorts are described in Table 4-17.

Overview of the Baltimore, MD cohort

Chromate production at the Baltimore, MD site began in 1845 and ultimately ceased in 1985 (Gibb et al., 2000b; Hayes et al., 1979). The original Baltimore cohort included workers who were newly employed between 1945 and 1974 (Hayes et al., 1979). The current cohort was defined by Gibb et al. (2000b) and excluded most workers who began work before August 1, 1950. This cutoff date coincided with when a new chromite ore mill and roasting plant were constructed, exposure mitigation measures were implemented, and extensive exposure information collection began (Gibb et al., 2015; Hayes et al., 1979). The vital status of 2357 workers were initially followed up through death or the end of 1992 (Gibb et al., 2000b) and then extended through 2011 for 2354 workers (Gibb et al., 2015) for a total of 91,186 person-years at risk. The mean duration of employment for the 2011 update of the cohort was 3.1 years and the mean number of years of follow-up was 38.9 years. The median duration of employment for the cohort was 0.4 years and the median number of years of follow-up was 39.9 years.

Gibb et al. (2000b) estimated Cr(VI) exposures for each person in each year based on job titles, the time spent in each sampling zone and exposure estimates based on ~70,000 contemporary measurements of Cr(VI) concentration in air during the study period. Samples included short-term air sampling in the workers' breathing zones from 1950–1961 followed by 24-hour routine measurements taken by 20 air samplers rotated through 154 fixed sites throughout the facility, and personal air sampling beginning in 1977. Exposure estimates were merged with work history data to estimate each workers' cumulative exposures during employment. All air measurements of hexavalent chromium were converted to units of mg CrO_3/m^3 as a common basis in Gibb et al. (2000b) because the prevailing regulatory standard was from the metric used by the U.S. Occupational Safety and Health Administration in its past Permissible Exposure Limits for chromic acid and chromates. The mean cumulative exposure[70] to CrO_3 reported in Gibb et al. (2015) Table 2 was 0.14 mg/m^3-years which converts to 72.8 $\mu g/m^3$-years of Cr(VI).[71] The 25th, 50th, and 75th percentiles were 0.52, 5.2, and 41.6 $\mu g/m^3$-years of Cr(VI). Company medical records provided smoking status at the beginning of employment for 91% of the cohort (Yes/No/Unknown); 74% smoked cigarettes, 16% did not smoke, and smoking status was unknown for 9%. No information on pack-years of smoking or how smoking status may have changed over time was available.

[70]Here the cumulative exposure is unlagged and untransformed.

[71]Conversion of mass of CrO_3 to mass of Cr(VI) is based on the contribution of the molecular weight (MW) of Cr to MW of CrO_3. Since the MW of Cr is 51.996 g/mol and the MW of CrO_3 is 99.99 g/mol, the conversion factor is 51.996/99.99 = 0.52. Units are further converted to $\mu g/m^3$ from mg/m^3 by multiplying by 1000 µg/mg.

Gibb et al. (2015) reported 217 deaths from lung cancer in this cohort compared to 133 expected deaths based on Maryland vital statistics for a SMR of 1.63 (95% CI: 1.42–1.86). The risk of lung cancer mortality was analyzed using a Cox proportional hazards model with age as the time variable and cumulative exposure as a time-varying covariate. In a model adjusted for smoking and age[72], each unit increase in $\log_{10}$ cumulative Cr(VI) exposure, lagged by 5 years, was associated with a 1.255-fold ($p < 0.001$) increase in the hazard ratio.

Gibb et al. (2020) re-analyzed this cohort with the same exposure and outcome data using a Cox proportional hazards model adjusted for smoking and age, but without log-transforming cumulative Cr(VI) exposure. In this analysis, untransformed cumulative Cr(VI) exposure, lagged by 5 years, was associated with a 1.64-fold (95% CI: 1.30, 2.04) increase in the hazard ratio. Gibb et al. (2020) also reported analyses of the untransformed cumulative Cr(VI) exposure using a conditional Poisson regression approach (Richardson and Langholz, 2012) to estimate the relative risk per unit of cumulative exposure (controlling for age and smoking) showing that cumulative Cr(VI) exposure, lagged by 15 years, was associated with a 1.82-fold (95% CI: 1.35, 2.45) increase in the hazard ratio.

Overview of the Painesville, OH cohort

The Painesville, OH chromate production plant was in operation from 1931–1972, with major renovations occurring in 1949–1950 and 1962–1964 to mitigate exposure and modernize plant operations (Proctor et al., 2004). Previous analyses of the Painesville plant relied on indirect measures of Cr(VI) in air, using measures of air total chromium and soluble/insoluble chromium dust measurements, and only studied workers employed prior to 1940 (Mancuso, 1997, 1975). The current cohort was defined by Proctor et al. (2016) to include workers employed after December 31, 1939. The vital statistics of 714 workers were followed up through death or the end of 2011 for a total of 24,535 person-years at risk. The mean duration of employment for the cohort was not explicitly reported, but falls within the interval of five to nine years (see Table 1 in Proctor et al. (2016) and the mean number of years of follow-up was 34.4 years.

The Proctor et al. (2016; 2004) studies obtained 800 measurements of airborne Cr(VI) from 23 historical industrial hygiene surveys for workers employed from 1940–1972. Using historical records of worker job histories over time and industrial hygiene data (which included Cr(VI) measurements), a job-exposure matrix (JEM) was constructed (Proctor et al., 2004). Usable data were available for 1943, 1945, 1948, 1957, and 1959–1971 (excluding 1962). Exposure estimates were merged with work history data to estimate each workers' cumulative exposures during employment. All Cr(VI) cumulative exposure estimates were reported in mg/m^3-years. The mean cumulative exposure to Cr(VI) was 1.1 mg/m^3-years (Proctor et al., 2016) which converts to 1.1×10^3 µg/m^3-years with a range of 0.2×10^3 µg/m^3-years to 22.1×10^3 µg/m^3-years. Employee records provided smoking status for 29% of the cohort (Yes/No/Unknown); of those, 22% smoked

[72]In this Cox proportional hazards regression, the time scale used was age and this controls for age in the model.

cigarettes, 7% did not smoke, and smoking status was unknown for 72%. No information on pack-years of smoking or how smoking status may have changed over time was available.

Proctor et al. (2016) reported 77 deaths from lung cancer in this cohort which yielded a SMR of 1.86 (95% CI: 1.45−2.28) compared to lung cancer mortality in Ohio and a SMR of 2.05 (95% CI: 1.59−2.50) compared to the U.S. population. Proctor et al. (2016) fit several models within the cohort and concluded that the linear Cox model with age as the time variable and controlling for smoking and age at hire had the best fit and reported a hazard ratio of 1.19 per mg/m^3-years increase in Cr(VI) exposure based on a regression coefficient of 0.17 per mg/m^3-years (95% CI: 1.11–1.27; p = 0.0006).

Table 4-17. Details of rationale for selecting a principal study on Cr(VI) for IUR derivation

Attribute	Preferred characteristics for candidate principal studies for the Cr(VI) IUR	Baltimore, MD Cohort	Painesville, OH Cohort
Study design characteristics	Sufficient follow-up time for outcomes to develop (this can depend on the health outcome being addressed). Study size and participation rates that are adequate to detect and quantify health outcomes being studied (without influential biases in study population selection) are preferred. Use of a study design or analytic approach that adequately addresses the relevant sources of potential confounding, including age, gender, and exposures to other risk factors for the outcome of interest.	Total person-time at risk: 91,186 person-years Size of cohort: 2354 workers Mean follow-up time: 38.7 years Confounding potential: Controlled for age and smoking; no mesothelioma deaths Effect modification potential: No known asbestos exposure and no mesothelioma deaths.	Total person-time at risk: 24,535 person-years Size of cohort: 714 workers Mean follow-up time: 34.4 years Confounding potential: Controlled for age and smoking; six mesothelioma deaths Effect modification potential: asbestos exposure is strongly indicated with six mesothelioma deaths.

Attribute	Preferred characteristics for candidate principal studies for the Cr(VI) IUR	Baltimore, MD Cohort	Painesville, OH Cohort
Relevance of exposure paradigm	Studies of chronic duration are preferred over studies of shorter exposure duration because they are most relevant to environmental exposure scenarios (potentially including both continuous exposure from ambient conditions and episodic activity-related exposures). When available studies observe effects across different ranges of exposures, studies that include relatively low exposure intensities that may represent conditions more similar to environmental exposures are preferred as there may be less uncertainty in extrapolation of those results to lower exposure levels.	Chronic duration Mean exposure Cr(VI): 72.8 µg/m^3-years. The 25th, 50th, and 75th% were 0.52, 5.2, and 41.6 µg/m^3-years	Chronic duration Mean exposure Cr(VI): 1.1×10^3 µg/m^3-years. Range from 0.2 µg/m^3-years to 22.1×10^3 µg/m^3-years
Measurement of exposure	Emphasis is placed on the specificity of exposure assessment in time and place with a preference for greater detail where possible. Exposure measurements that are site and task specific provide generally preferred exposure information. Where available, individual-level measurements are generally preferred. Measurement techniques that are more specific to the agent of concern are preferred over less specific analytical methods. Better characterization of airborne concentrations is preferred. Stronger studies will often be based upon knowledge of individual work histories (job titles/tasks with consideration of changes over time); however, appropriate group-based exposure estimates may also be relevant. Exposure reconstruction and estimating exposures based on air sampling from other time periods and/or operations are less preferred methods of exposure estimation.	~70,000 measurements during 1950–1974. Early samples were short-term air samples in the workers' breathing zones, later 24-hours samples from 154 fixed sites, and full-shift personal air sampling began in 1977. Sampling records for 9 years could not be located (1950–56, 1960–61) and those values were imputed based on existing data to model those job-specific exposure values. Individual work histories matched to job-specific exposure estimates based on sampling measurements.	800 measurements during 1940–1972. No personal samples. Uncertainty in short-term workers' exposures: Proctor et al. (2004) "company records lacked sufficient information on these individuals to reconstruct their work histories." Individual work histories matched to job-specific exposure estimates based on sampling measurements.
Measurement of covariates	Studies that considered the potential effects of confounding by relevant covariates are preferred over those without such consideration—unless confounding is not a major concern.	Age is well measured. Smoking status was identified for 93% of the cohort.	Age is well measured. Smoking status was identified for 28% of the cohort.

Attribute	Preferred characteristics for candidate principal studies for the Cr(VI) IUR	Baltimore, MD Cohort	Painesville, OH Cohort
Measurement of effect(s)	Cancer incidence data are generally preferred over cancer mortality data (U.S. EPA, 2005a). In the absence of cancer incidence data, cancer mortality data are appropriate with preference for cause of death classified using international classification disease (ICD) codes at time of death.	Lung cancer data were obtained from death certificates. 217 lung cancer cases. No deaths from mesothelioma and no evidence of outcome misclassification.	Lung cancer data were obtained from death certificates. 77 lung cancer cases. 3 deaths from mesothelioma (of 6 total) were initially classified as lung cancer deaths
Analysis methodology	Studies conducting and reporting regression results of within cohort comparisons and those with β and SE(β) are preferred over standardized mortality ratio (SMR) results. Occasionally studies reporting standardized rate ratio (SRR) or SMR results with sufficient specificity by exposure category may allow for post hoc estimation of β and SE(β)—although if the lowest exposure category is defined by the lowest quantile/category of exposure, such estimates may be biased towards the null.	Analyses included multiple model forms (types of regression) with multiple parameterizations of covariates and lags for exposure.	Analyses included multiple model forms (types of regression) with multiple parameterizations of covariates and lags for exposure.

Table 4-17 summarizes key considerations related to study attributes that were considered in the rationale for identifying the principal cohort. The Baltimore, MD cohort was (1) larger than the Painesville cohort, (2) had longer follow-up time, (3) had more deaths from lung cancer, (4) had no deaths from mesothelioma, despite having 66,651 additional years of person-time at risk than in the Painesville cohort, suggesting lower potential for confounding by asbestos exposure, (5) had more than an order of magnitude lower average exposures which can be more relevant to estimating effects at lower exposures and requires less extrapolation, (6) had more air samples to estimate exposures, and (7) had more complete data on smoking. EPA selected the Baltimore, MD cohort as the basis for deriving the IUR.

4.4.2. Dose-Response Analysis—Adjustments and Extrapolations Methods

The first step towards deriving an inhalation unit risk for lung cancer was to identify candidate effect estimates (i.e., beta coefficients from the regression analyses) from studies of the principal cohort. Once the lung cancer effect estimates have been obtained, they are adjusted for differences in air volumes between workers and other populations due to exposure frequency and breathing rates. Conversions between occupational Cr(VI) exposures and continuous environmental exposures were made to account for differences in the number of days exposed per year, and in the amount of air inhaled per day. Those adjusted values can be applied to the U.S. population as a whole in EPA life-table analyses. These life-table analyses allow for the estimation

of an exposure concentration associated with a specific extra risk of cancer incidence caused by inhalation of Cr(VI); the specific extra risk is called the benchmark response (BMR) and a value of 1% is standard for cancer outcomes in people. Those exposure concentrations serve as points of departure (POD) from which IURs can be extrapolated. Non-occupational exposure adjustment and methods applied for the life-table analysis are described in detail in Section 4.4.3.

Because a mutagenic mode of action for Cr(VI) carcinogenicity (see Section 3.2.3) is "sufficiently supported in (laboratory) animals" and "relevant to humans," EPA used a linear low dose extrapolation from the POD in accordance with Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a).

4.4.2.1. *Cancer risk models for Cr(VI) inhalation exposures*

A cancer risk model predicts the probability of cancer in an individual with a specified history of exposure to a cancer-causing agent. In the case of inhalation exposure to Cr(VI), the lung cancer effects are of chief concern, and workers' individual cumulative exposure to Cr(VI) are used to predict cancer risk. Different types of regression analyses were used to model the lung cancer effect of Cr(VI) in the Baltimore, MD cohort. The model forms are described below.

The Cox proportional hazards model (Cox, 1972) is one of the most commonly used statistical models for the epidemiologic analysis of survival and mortality in cohort studies with extensive follow-up, including studies of the Baltimore, MD cohort (Gibb et al., 2020; Gibb et al., 2015; Gibb et al., 2000b). The Cox proportional hazards model assumes that a function of covariates (e.g., exposures) result in hazard functions that are a constant proportion of the baseline hazard function in unexposed individuals over some timescale, typically calendar time or age (e.g., the background age-specific rates of lung cancer in the population). One of the strengths of this model is that knowledge of the baseline hazard function is not necessary, and no particular shape is assumed for the baseline hazard; rather, it is estimated nonparametrically.

Another methodology used to analyze the Baltimore, MD cohort (Gibb et al., 2020) was the conditional Poisson regression approach proposed by Richardson and Langholz (R&L) to estimate the relative risk per unit of cumulative exposure (Richardson and Langholz, 2012). The R&L approach maximizes a conditional likelihood expression that allows for covariates like age and smoking to be included in the model, but avoids estimation of all the stratum-specific parameters by treating them as nuisance terms. This property is made possible by separating and then cancelling the nuisance terms in the likelihood function. Thus, the R&L approach models the effects of age and smoking when estimating the effect of Cr(VI), but does not yield the specific effect estimates for age and smoking.

4.4.2.2. *Cancer risk parameters*

The Cox regression results from the Baltimore, MD cohort are shown in Table 4-18.

Table 4-18. Results of Cox proportional hazards modeling of cumulative chromium exposure (mg CrO_3/m^3-years) by different lag periods (age and smoking are included in model). Table adapted from Table 1 of Gibb et al. (2020).

Lag period (y)	β per mg CrO_3/m^3-year	SE(β)	Hazard ratio Exp(β)	95% CI (β)	−2 log(L)
0	0.4712	0.1133	1.60	1.28-2.00	2830.23
5	0.4868	0.1145	1.63	1.30-2.04	2829.80
10	0.4939	0.1197	1.64	1.30-2.07	2830.52
15	0.4812	0.1333	1.62	1.25-2.10	2833.03

Note: 1 mg CrO_3 = 0.520 mg Cr(VI); CrO_3/m^3-year = (CrO_3/m^3)(year).

The measure of fit (−2 Log(L)) of the Cox proportional hazards models of the lung cancer risk adjusted for age and smoking were very similar for all lag periods, although the fit for the 5-year lag was slightly better than for the other lags—although not statistically better. The rationale for the lag period is that there is often a latency period for cancer beginning with the initial incidence of cancer and extending to the time of cancer mortality. In this conceptual model, the exposures that are experienced by the individual after cancer has begun are no longer expected to cause lung cancer, and thus those exposures may not be etiologically relevant. Here the results show little difference in effect size across the different lag times. This is likely due to the fact that exposures ceased in 1982 and follow-up continued until 2011 so there was little difference in lagged and unlagged exposures. Section 4.4.5 provides a sensitivity analysis across the different lag lengths.

The lung cancer effect estimate for the 5-year lag in Table 4-18 above (Gibb et al., 2020) is in units of per mg CrO_3/m^3-year and was converted to unit of per μg Cr(VI)/m^3-year as follows:

$$1 \text{ mg } CrO_3/m^3\text{-year} \bullet [0.52 \text{ mg Cr(VI)/mg } CrO_3] \bullet [1000 \text{ μg/mg}] = 520 \text{ μg Cr(VI)}/m^3\text{-year}$$

$$5\text{-year lag } \beta_{Cr(VI)} = 0.4868 \text{ per mg } CrO_3/m^3\text{-year} = 0.4868/(1 \text{ mg } CrO_3/m^3\text{-year})$$

$$= 0.4868/(520 \text{ μg Cr(VI)}/m^3\text{-year})$$

$$= 9.362 \times 10^{-4} \text{ per μg Cr(VI)}/m^3\text{-year}$$

The inhalation unit risk is derived from the one-sided 95th% upper bound of β. Gibb et al. (2020) reported a two-sided 95% confidence interval as is the standard practice in the epidemiologic literature (i.e., from the 2.5th% to the 97.5th% bounds). EPA estimated the one-sided 95th% upper bound (UB) of β by assuming the distribution of β was normally distributed (which is appropriate for the Cox Proportional Hazards model) as follows:

$$\text{One-sided 95th\% UB of } \beta = \beta + 1.645(se(\beta))$$

= 0.4868 per mg CrO_3/m^3-year +1.645 • (0.1145 per mg CrO_3/m^3-year)
= 0.6752 per mg CrO_3/m^3-year
= (0.6752 per mg CrO_3/m^3-year) / (520 µg Cr(VI)/m^3-year)
= 1.298×10^{-3} per µg Cr(VI)/m^3

This one-sided 95th% upper bound of β from the Cox Proportional Hazards analysis in Gibb et al. (2020) will be used to derive an estimate of the IUR using a life-table analysis.

R&L regression results from the Baltimore, MD cohort are shown in Table 4-19.

Table 4-19. Results for relative exponential exposure-response (R&L) model adjusted for age and smoking. Table adapted from Table 2 of Gibb et al. (2020).

# Age groups[a]	Lag period (y)	β	SE(β)	*RR* = exp(β)	95% CI(β)	−2 log(L)
1	0	0.454	0.098	1.57	1.30–1.91	9283.51
	5	0.454	0.098	1.57	1.30–1.91	9283.62
	10	0.451	0.101	1.55	1.29–1.91	9286.50
	15	0.414	0.108	1.51	1.22–1.87	9291.89
2	0	0.454	0.098	1.57	1.30–1.91	9283.50
	5	0.461	0.098	1.59	1.31–1.92	9282.79
	10	0.463	0.100	1.59	1.31–1.93	9284.08
	15	0.474	0.107	1.60	1.30–1.98	9286.46
3	0	0.915	0.047	2.50	2.28–2.74	8854.75
	5	0.933	0.048	2.59	2.31–2.79	8846.57
	10	0.982	0.050	2.67	2.42–2.94	8845.78
	15	1.088	0.056	2.97	2.66–3.31	8848.71
4	0	0.506	0.133	1.66	1.28–2.15	4327.08
	5	0.522	0.133	1.69	1.30–2.19	4326.07
	10	0.548	0.139	1.73	1.32–2.27	4325.97
	15	0.599	0.152	1.82	1.35–2.45	4325.95
5	0	1.179	0.036	3.25	3.03–3.49	8153.85
	5	1.246	0.036	3.48	3.24–3.73	8091.17
	10	1.387	0.040	4.00	3.70–4.33	8035.39
	15	1.559	0.044	4.75	4.36–5.18	8030.41
6	0	1.142	0.036	3.13	2.92–3.36	8253.33
	5	1.164	0.036	3.20	2.98–3.44	8235.51
	10	1.200	0.038	3.39	3.08–3.58	8238.56
	15	1.375	0.043	3.95	3.64–4.30	8223.38

Note: 1 mg CrO_3 = 0.520 mg Cr(VI).

[a]One age group (all ages, 15-96); two age groups (≥15 to 65 and ≥65); three age groups (ages ≥15 to 60, ≥60 to ≥70); four age groups (≥15 to 60, ≥60 to 65, ≥65 to 75, and ≥75); five age groups (ages ≥15 to 60, ≥60 to 65, ≥65 to 70, ≥70 to 75, and ≥75);six age groups (ages ≥15 to 55, ≥55 to 60, ≥60 to 65,≥65 to 70, ≥70 to 75, and ≥75).

The R&L analysis based on four age groups fit the Baltimore, MD cohort better than the analyses based on other numbers of age groups as evidenced by the lower fit statistics, and within the 4-age group analysis, the fits were very similar for all lag periods, although the fit for the 15-year lag was slightly better than for the other lags—although not statistically better. Section 4.4.5 provides a sensitivity analysis across the different lag lengths.

The lung cancer effect estimate for the 15-year lag in Table 2 from Gibb et al. (2020) is 0.599 per mg CrO_3/m^3-year and was converted to unit of per µg Cr(VI)/m^3-year as follows:

$$1 \text{ mg } CrO_3/m^3\text{-year} \bullet [0.52 \text{ mg Cr(VI)/mg } CrO_3] \bullet [1000 \text{ µg/mg}] = 520 \text{ µg Cr(VI)}/m^3\text{-year}$$

$$5\text{-year lag } \beta_{Cr(VI)} = 0.599 \text{ per mg } CrO_3/m^3\text{-year} = 0.599/(1 \text{ mg } CrO_3/m^3\text{-year})$$

$$= 0.599/(520 \text{ µg Cr(VI)}/m^3\text{-year})$$

$$= 1.152 \times 10^{-3} \text{ per µg Cr(VI)}/m^3\text{-year}$$

$$\text{One-sided 95th\% UB of } \beta = \beta + 1.645(se(\beta))$$

$$= 0.599 \text{ per mg } CrO_3/m^3\text{-year} + 1.645 \bullet (0.152 \text{ per mg } CrO_3/m^3\text{-year})$$

$$= 0.849 \text{ per mg } CrO_3/m^3\text{-year}$$

$$= (0.849 \text{ per mg } CrO_3/m^3\text{-year}) / (520 \text{ µg Cr(VI)}/m^3)$$

$$= 1.633 \times 10^{-3} \text{ per µg Cr(VI)}/m^3$$

This one-sided 95th% upper bound of β from the R&L analysis in Gibb et al. (2020) will be used to derive an estimate of the IUR using a life-table analysis.

4.4.3. Inhalation Unit Risk Derivation

4.4.3.1. *Life-table analysis to derive an IUR*

The β coefficients (slopes) for lung cancer risks attributable to cumulative exposures to Cr(VI) from the Gibb et al. (2020) are used in life-table analyses to predict the risk of cancer as a result of the exposure over a lifetime. The life-table analysis divides a lifetime into small age-specific intervals and sums the risks of lung cancer incidence in each age group in the presence and absence of Cr(VI) exposure. This is done to assess the age-specific risk of lung cancer incidence while accounting for competing causes of death. The lung cancer risk in a particular year of life is conditional on the assumption that the individual is alive, and at risk of incident lung cancer, at the start of the year for each age-specific interval. Consequently, the risk of a Cr(VI)-related lung cancer within a specified year of life is calculated as a function of (1) the probability of being alive at the start of the year, (2) the background probability of getting lung cancer, and (3) the increased risk of getting lung cancer from Cr(VI) exposure within the specified year. The lifetime risk is then the sum of all the yearly risks. This procedure is performed to calculate the lifetime risk both for an unexposed individual (R0) and for an individual with exposure to Cr(VI) (Rx).

"Extra risk" for lung cancer is a calculation of risk which adjusts for background incidence rates of lung cancer, by estimating risk at a specified exposure level and is calculated as follows (U.S. EPA, 2012a):

$$\text{Extra Risk} = (Rx - R0) / (1 - R0)$$

The inhalation unit risk (IUR) is the risk of incident lung cancer per unit concentration (μg/m^3) in inhaled air. The unit risk is calculated by using life-table analysis to find the exposure concentration (EC) that yields a 1% (0.01) extra risk of lung cancer. The 1% value is referred to as the Benchmark Response (BMR). This 1% value is used because lung cancer is a severe adverse effect and 1% also represents a lung cancer response level that is near the low end of the observable range (U.S. EPA, 2012a). This is also consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b), which notes that a BMR of 1% is typically used for epidemiological data since higher values may involve upward extrapolation.

Because a mutagenic mode of action for Cr(VI) carcinogenicity (see Section 3.2.3) is "sufficiently supported in (laboratory) animals" and "relevant to humans," EPA used a linear low dose extrapolation from the POD in accordance with Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a). Given the EC associated with a 1% extra risk (EC_{01}), the unit risk is the slope of a linear exposure-response line from the origin through the EC_{01}:

$$\text{Unit Risk} = 0.01 / EC_{01}$$

A unit risk value may be calculated based on both the best estimate (β) and the one-sided 95% upper confidence bound (UB) on the best estimate. The value based on the one-sided upper 95% confidence bound is normally used for decision-making, since it corresponds to a one-sided lower 5% confidence bound (LB) on the exposure level yielding 1% extra risk (LEC_{01}).

$$\text{IUR} = 0.01 / LEC_{01}$$

Life-table calculations require as input the all-cause mortality rates and lung cancer incidence rate for the general U.S. population in each year of life. The all-cause mortality data were obtained from the National Vital Statistics Report Vol 68 No 7 Table 1 (Arias et al. (2017), which provides data from the U.S. population in 2017. Lung cancer incidence rates were obtained by downloading 2017 data for malignant neoplasms of bronchus and lung (ICD-10 C33-C34) from CDC WONDER[73]. Because cause-specific rates were given for 5-year intervals, the cause-specific rate for each 5-year interval was applied to each age within the interval.

The detailed equations for calculating lifetime excess cancer risk for a specified exposure concentration in the presence of competing risks are based on the approach used by NRC (1988) for evaluating lung cancer risks from radon. The equations are detailed in Appendix E. The SAS code for

[73] http://wonder.cdc.gov/ucd-icd10.html.

lung cancer life-table analysis was provided to EPA by NIOSH[74] and was adapted for use by (1) entering the data noted above; (2) adding adjustment factors to account for differences between occupational exposures and non-occupational exposure; (3) adding an equation to compute extra risk; and (4) adding a macro to solve for the EC_{01} or the LEC_{01}. The SAS codes for performing the lung cancer life-table calculations are provided in Appendix E.

The adjustment factors to account for differences between occupational exposures and non-occupational exposure follow EPA guidance (U.S. EPA, 2009) that acknowledges there are differences in breathing rates between workers (10 m^3 per 8-hour day) and non-workers (20 m^3 per 24-hour day) and that workers are exposed 240 days per year while non-workers are exposed 365 days per year (U.S. EPA, 2016c, 2014e, 2012d, 2011d). Thus, a worker is assumed to inhale 2,400 m^3 of workplace air per year while a non-worker is assumed to inhale 7,300 m^3 of air per year. Since the effect estimates for Cr(VI) effects on lung cancer risks are in terms of 'per occupational year', the life-table procedure adjusts for the differences in air volume breathed per year to represent non-occupational exposures.

4.4.3.2. *Summary of lifetime unit risk estimates—not accounting for assumed increased early-life susceptibility*

The derivation of the unit risk—not accounting for assumed increased early-life susceptibility—is based upon the two main regression modeling results in Gibb et al. (2020): (1) the Cox Proportional Hazard model with exposure lagged by 5 years, and (2) the R&L model with four age groups and exposure lagged by 15 years. Note that this estimate of the unit risk is based on the assumption that the relative risks or hazard ratios are independent of age.

Table 4-20. Calculation of lifetime cancer unit risk estimate not accounting for assumed increased early-life susceptibility

Source	Table in original publication	β (Slope) per mg CrO_3/m^3		β (Slope) per µg Cr(VI)/m^3		Exposure Concentration associated with BMR (1% Extra Risk) [µg Cr(VI)/m^3]		Lifetime Unit Risk [per µg Cr(VI)/m^3]	
		MLE	95% UB	MLE	95% UB	EC_{01} MLE	LEC_{01} 5% LB	MLE	95% UB
Gibb et al. (2020) Cox PH Model	Table 1 5-year lag	0.487	0.675	9.36×10^{-4}	1.30×10^{-3}	1.25	0.899	8.02×10^{-3}	1.11×10^{-2}
Gibb et al. (2020) R&L Model	Table 2 4 age groups 15-year lag	0.599	0.849	1.15×10^{-3}	1.63×10^{-3}	1.35	0.952	7.41×10^{-3}	1.05×10^{-2}

[74]Beta Version. SAS 30NOV18, provided by Randall Smith, National Institute for Occupational Safety & Health.

The results from the Cox model yielded an estimate of the lifetime unit risk of 1.11×10^{-2} per µg Cr(VI)/m^3 while the results from the R&L model yielded an estimate of the lifetime unit risk of 1.05×10^{-2} per µg Cr(VI)/m^3. These two estimates are very close to each other and thus mutually support one another. EPA advanced the estimate of the lifetime unit risk derived from the Cox proportional hazards models with an exposure lag of 5 years for the following reasons: (1) the Cox proportional hazards model is a well-established method for epidemiological analyses that is commonly used in cohort studies, and (2) the results from this type of model have been used as the basis for EPA IRIS IUR derivations for lung cancer (U.S. EPA, 2014e), breast cancer (U.S. EPA, 2016c) and lymphohematopoietic cancer (U.S. EPA, 2016c). In the absence of evidence of early-life susceptibility, the lifetime unit risk for lung cancer caused by inhalation exposure to Cr(VI) is considered to be best estimated as 1.11×10^{-2} per µg Cr(VI)/m^3.

Because a mutagenic mode of action for Cr(VI) carcinogenicity (see Section 3.2.3) is "sufficiently supported in (laboratory) animals" and "relevant to humans," and as there are no chemical-specific data to evaluate the differences between adults and children, increased early-life susceptibility should be assumed. If there is early-life exposure, age-dependent adjustment factors (ADAFs) are applied, as appropriate, in accordance with the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). See Section 4.4.4 below for more details on the application of ADAFs.

4.4.4. Application of age-dependent adjustment factors

The derivation of the IUR when increased early-life susceptibility should be assumed is based on the same main Cox proportional hazards regression modeling results with exposure lagged by 5 years (Gibb et al., 2020). The process for deriving an IUR when increased early-life susceptibility should be assumed involves an initial estimation of a unit risk based only on adult-only exposures (U.S. EPA, 2016c), followed by the application of age-dependent adjustment factors to age-specific risks for children under age 16 years, and a summary of risks across all ages weighted by the age-dependent adjustment factors. This is accomplished with several steps.

- The first step is to apply the effect estimate (i.e., the MLE β) from the Baltimore, MD cohort and the 95% UB in a life-table initiating exposures at 16 years of age—instead of at birth. This process estimates the unit risks for the 54-year period between age 16 years and age 70 years (IRIS' assumption of a lifetime).
- The values of the EC01 and LEC01 are derived in the same way using the life-table procedure.
- These EC01 and LEC01 values are then divided into the benchmark response of 1% to compute the 'adult-exposure-only' unit risk estimates.
- The 'adult-exposure-only' unit risk estimates are multiplied by 70/54 to rescale the 54-year adult period to 70 years. This yields the 'adult-based' lifetime unit risk.

- The last step is to apply the ADAFs which adjust the 'adult-based' lifetime age-specific unit risk for children ages less than two years upwards by 10-fold during those years of life, and the unit risk for children ages 2–15 upwards by 3-fold during those years of life, and then applies the unadjusted 'adult-based' lifetime unit risk for people aged 16–70 during those years of life. The weighted sum of these three partial unit risks is the ADAF-adjusted lifetime IUR.

Table 4-21. Calculation of total cancer unit risk estimate from adult-only exposure

Source	β (Slope) per µg Cr(VI)/m³		Exposure Concentration associated with BMR (1% Extra Risk) Starting exposure at age 16 years [µg Cr(VI)/m³]		Adult-exposure-only Unit Risk [per µg Cr(VI)/m³] (54 years)		Adult-based Unit Risk [per µg Cr(VI)/m³] (70 years)	
	MLE	95% UB	EC_{01}(16+) MLE	LEC_{01}(16+) 5% LB	MLE	95% UB	MLE	95% UB
Gibb et al. (2020) Cox PH Model 5-year lag	9.36×10^{-4}	1.30×10^{-3}	1.64	1.18	6.12×10^{-3}	8.48×10^{-3}	7.93×10^{-3}	1.10×10^{-2}

The results from the Cox model yielded an estimate of the 'adult-based' unit risk of 1.10×10^{-2} per µg Cr(VI)/m³. Application of the ADAFs to the 'adult-based' (rescaled as discussed above) unit risk estimate for Cr(VI) for a lifetime inhalation exposure scenario is presented below. The unit risk for each age group is the product of the values for the ADAF, the adult-based unit risk, and the duration adjustment in columns 2–4 [e.g., $10 \times (1.10 \times 10^{-2}) \times 2/70 = 3.14 \times 10^{-3}$], and the total risk is the sum of the partial risks. This lifetime inhalation unit risk estimate for a constant exposure of 1 µg Cr(VI)/m³ is adjusted for potential increased early-life susceptibility, assuming a 70-year lifetime.

Table 4-22. Total cancer risk from exposure to constant Cr(VI) exposure level of 1 µg/m³ from ages 0–70 years, adjusted for potential increased early-life susceptibility

Age Group	ADAF	Adult-based unit risk (per µg Cr(VI)/m³)	Duration adjustment	Unit risk [per µg Cr(VI)/m³]
0–<2 years	10	1.10×10^{-2}	2 years/70 years	3.14×10^{-3}
2–<16 years	3	1.10×10^{-2}	14 years/70 years	6.60×10^{-3}
≥16 years	1	1.10×10^{-2}	54 years/70 years	8.49×10^{-3}
			Total Lifetime Risk	**1.82×10^{-2}**

The lifetime inhalation unit risk for Cr(VI) is 1.82×10^{-2} per µg Cr(VI)/m³. This value is rounded to 2×10^{-2} per µg Cr(VI)/m³.

If calculating the cancer risk for a 30-year exposure to a constant average concentration of 0.01 µg hexavalent chromium/m^3 from ages 0 to 30 years, the duration adjustments would be 2/70, 14/70, and 14/70, and the partial risks would be ($10 \times 0.011 \times 0.01 \times 2/70 = 3.1 \times 10^{-5}$), ($3 \times 0.011 \times 0.01 \times 14/70 = 6.6 \times 10^{-5}$), and ($1 \times 0.011 \times 0.01 \times 14/70 = 2.2 \times 10^{-5}$), resulting in a total risk estimate of 1.2×10^{-4}.

If calculating the cancer risk for a 30-year exposure to a constant average average concentration of 0.01 µg hexavalent chromium/m^3 from ages 20 to 50 years, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks would be 0, 0, and ($1 \times 0.011 \times 0.01 \times 30/70 = 4.7 \times 10^{-5}$), resulting in a total risk estimate of 4.7×10^{-5}.

4.4.5. Uncertainties in the Derivation of the Inhalation Unit Risk

Several potential sources of uncertainty were identified in the derivation of the Cr(VI) inhalation unit risks. As discussed below, these were not found to be major influences in this evaluation—including two potential sources of uncertainties generally associated with larger uncertainty (model uncertainty and low dose extrapolations).

Sources of uncertainty in this assessment are outlined below.

4.4.5.1. *Uncertainty in exposure assessment*

Routine air sampling was initiated after construction of the new Baltimore, MD facility in 1950 and followed written documentation specifying strategies for air sampling. Sampling was intended to represent the "typical/usual exposures" to workers (Gibb et al., 2000b). Table 4-23 below details the sampling regimen over time. In constructing the job-exposure-matrix to assign individual exposure for each worker, Gibb et al. (2000b) relied on approximately 70,000 measurements across the study period. While the sampling regimes changed over time and can reasonably be expected to have improved in quantity and specificity, the samples were collected methodically and used the same analytical method for assessing Cr(VI) concentration in dust over the study period (Gibb et al., 2000b).

These exposure estimates were used to construct a job-exposure-matrix (JEM) for each of the 114 job titles in each of the 36 years of the study period. According to Gibb et al. (2000b), the JEM was "virtually complete" for the later years (1971–1985) and "fairly complete" for the early years from 1950–1956 and 1960–1961. While the sampling records for nine years could not be located, those values were imputed based on existing data to model those job-specific exposure values. EPA considered uncertainty to be low for the 24 out of 36 years when sampling records was available and low-to-medium for the missing years that were bookended by actual sampling values. As exposures may reasonably be assumed to have decreased over the study period as industrial hygiene practices improved, the interpolation between higher and lower exposure periods was likely to have captured those interim exposure concentrations.

Table 4-23. Overview of air sampling program for the Baltimore cohort evaluated by Gibb et al., (2015; 2000b)

Exposure measurement system	Years implemented	Frequency and duration
Airborne dust via high-volume air sampling pumps and impingers, with sampling wand held in worker breathing zone.	1950–1961	Short-term samples (tens of minutes).
24-hour routine measurements (fixed-site monitors) using 20 tape air samplers (Research Appliance Co., Allison Park, PA). Observation of how much workers spent in the vicinity of each of these monitors.	Mid-1960s–1979	24 1-hour samples. Samplers rotated through 154 fixed sites representing exposure zones.
	1979–1985	After 1979, frequency reduced to 8 3-hour samples, and number of fixed sites reduced to 27.
Routine personal sample collection using NIOSH standard method P and CAM 169 (NIOSH, 1972).	1977–1985	Full-shift sampling.

4.4.5.2. *Uncertainty in the exposure metric*

Gibb et al. (2000b) fit multiple models of lung cancer risks using untransformed and transformed cumulative exposure to Cr(VI) with log base-10 transformed Cr(VI) providing the better overall model fit. Gibb et al. (2015) also reported updated lung cancer results based on log base-10 of cumulative exposure to Cr(VI). While log transformation of concentration-based cumulative exposure is commonplace in epidemiological analyses because those concentrations are often log-normally distributed, risk calculation based on log-transformed exposure suffer from exposure-response irregularities such as zero risk whenever the exposure has a numerical value of one (in any units) [i.e., log10(1) = 0 or ln(1) = 0], and when risks are extrapolated below one unit of exposure, the sign of the risk estimate flips from positive to negative such that lower exposure appears to be health protective as an artifact of the transformation. For the purpose of estimating an IUR, exposure-response results in terms of untransformed cumulative exposures to Cr(VI) can be more useful than log-transformed exposures. Gibb et al. (2020) reported risks of lung cancer associated with untransformed cumulative Cr(VI). While a transformed exposure may provide a better overall model across the entire range of exposures in a study, as in the case of Gibb et al. (2020), those model results did not meet the needs for estimating an IUR based on a POD in the low exposure range, and thus EPA selected the results from the models based on untransformed cumulative Cr(VI)—even if there is some uncertainty concerning the relative fits of different exposure metrics.

The two candidate IUR's are based on the same cohort that was most highly rated and preferred on the majority of additional considerations for exposure-response, there are some aspects of the specific modeling details that were further considered in order to judge their potential impact on the IUR. Specifically, the exposure lags and the number of age groups that yielded the better overall fits, often the fit differences were small enough so as to be essentially equal in fit. Three additional sets of candidate unit risks were derived to show the differences in

those values had those combinations been selected instead, and to allow for comparison between the two candidate IURs on a common basis of exposure lag length.

Table 4-24. Variation in unit risks among the Cox Proportional Hazards model results by lag length

Cox Proportional Hazards	Lag period (y) in Gibb et al. (2020) matched in life-table	
Lag period (y) in Gibb et al. (2020)	Lifetime Unit Risk (95%UB) without ADAFs [per µg Cr(VI)/m^3]	Lifetime Unit Risk (95%UB) with ADAFs [per µg Cr(VI)/m^3]
0	1.16×10^{-2}	2.00×10^{-2}
5	1.11×10^{-2}	1.82×10^{-2}
10	1.05×10^{-2}	1.64×10^{-2}
15	9.82×10^{-3}	1.47×10^{-2}

4.4.5.3. *Uncertainty in the outcome metric*

Lung cancer mortality was ascertained from death certificates according to specific codes from the International Classification of Diseases—eighth edition, and this coding system and those of previous editions have been stable over time. Uncertainty is considered to be very low for lung cancer mortality.

4.4.5.4. *Uncertainty due to length of follow-up*

There is little potential uncertainty regarding the length of follow-up for cancer mortality. The hire dates among this cohort ranged from August 1, 1950 to December 31, 1974 (the mean date of hire was mid-1957) (Gibb et al., 2000b). Follow-up continued until the date of death, age 96 years, or December 31, 2011, whichever occurred first. Therefore, the range of follow-up was from 37 to 61 years, with a mean of more than 38 years.

4.4.5.5. *Uncertainty in model form*

For lung cancer mortality, the Cox proportional hazards model is a well-established method for epidemiological analyses that is commonly used in cohort studies because this type of survival analysis takes into account differences in follow-up time among the cohort and is approximately linear at low exposures. This model form allows for the evaluation and control of important potential confounding factors such as age and smoking, and for the modeling of exposure as a continuous variable. There is little uncertainty in the choice of model form. Additionally, the R&L model is an alternative approach to the Poisson model and results from this modeling yielded similar results which further reduces the uncertainty in the choice of model form.

4.4.5.6. *Uncertainty in control of potential confounding in modeling lung cancer mortality.*

It is well known that smoking is a strong independent risk factor for lung cancer. Company medical records provided smoking status at the beginning of employment for 91% of the cohort (Yes/No/Unknown); 74% smoked cigarettes, 16% did not smoke, and smoking status was unknown for 9% (Gibb et al., 2000b). No information on pack-years of smoking or how smoking status may have changed over time was available. As an important potential confounder of the lung cancer mortality analysis, smoking was controlled for in the analyses of lung cancer mortality associated with exposure Cr(VI) (Gibb et al., 2020; 2015; 2000b). Each of the Cox proportional hazards analyses showed that smoking at the beginning of employment was a strong predictor of lung cancer risk. While additional information on the cumulative exposure to smoking may have been helpful to more completely control for smoking, it is clear that as measured, smoking was a strong independent predictor of lung cancer risks and was independent of cumulative Cr(VI) exposure as it was measured at the beginning of employment. There remains some low uncertainty as to any potential residual confounding that might be attributed to lack of smoking data on 9% of the cohort and the lack of information on any changes in smoking over time. However, the Baltimore cohort had much better data on smoking compared to the Painesville cohort, and thus the selection of the Baltimore cohort minimizes the potential for confounding by smoking among the available cohorts.

4.4.5.7. *Uncertainty due to potential effect modification*

Among the 217 deaths from lung cancer in workers, only four were among nonsmokers (Gibb et al., 2015) and the investigators were unable to evaluate any potential statistical interaction between smoking and Cr(VI) exposure. It is theoretically possible that the risk of lung cancer mortality estimated in this current assessment is a reflection of a positive synergy between smoking and Cr(VI), and that the adverse effect of Cr(VI) among nonsmokers has been overestimated. However, this possibility cannot be assessed and remains an uncertainty. The unit risk of the lung cancer risk herein would be health protective for any population that had a lower prevalence of smoking than that of the Baltimore cohort.

4.4.5.8. *Uncertainty in low dose extrapolation*

A common source of uncertainty in quantitative cancer risk assessments generally derives from extrapolating from high doses in animals to low doses in humans. Compared to assessments based on animal data, the uncertainty from low-dose extrapolation in this assessment, which uses occupational epidemiology data, is considered to be low because the POD was well within the range of observed exposure data. The POD for lung cancer was based on 1% extra risk and yielded an LEC_{01} of 0.899 µg Cr(VI)/m^3 from the Cox analysis and 0.951 µg Cr(VI)/m^3 from the R&L analysis. Table 2 of Gibb et al. (2015) shows that the median cumulative exposure to CrO_3 was 0.01 mg CrO_3/m^3-years and the 25%-tile of CrO_3 was 0.001 mg CrO_3/m^3-years, and the minimum was zero.

Converting to µg Cr(VI)/m^3, the median was 52 µg Cr(VI)/m^3 and the 25%-tile was 5.2 µg Cr(VI)/m^3. Here the PODs appear to be between the minimum and the 25%-tile and thus not outside the range of observed exposures. Thus, there is little uncertainty in extrapolation of the risk function below the POD associated with a 1% BMR.

4.4.5.9. *Uncertainty in extrapolation of findings in adults to children.*

The analysis of lung cancer mortality using the Cox proportional hazards model assumed that the effect was independent of age, while the analysis using the R&L approach allowed for effects to be different by age group—although this analysis did not provide any estimates of what the age effect was beyond showing that the relatively younger cohort members appeared to be at higher risk of lung cancer mortality than the older cohort members. Given that both of these analyses yielded approximately the same estimate of the IUR, it appears that while there may be an age-related effect of Cr(VI) exposure on the risk of lung cancer, two different analyses that treated age differently yielded essentially the same unit risk when the life-table analysis assumed that the effect was independent of age.

However, Cr(VI) was found to cause cancer by a mutagenic mode of action, and chemical-specific data are not available to address early-life exposure. According to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), ADAF are applied for children and risks were based on application of age-dependent risk modifiers of an "adult-only" unit risks such that effect were independent of age among people age 16 years and older. There is some uncertainty that these default ADAF would be health-protective of children although this uncertainty is considered to be low.

The inhalation unit risk (IUR) is a plausible upper bound on the estimate of risk per µg/m^3 air breathed. The IUR can be multiplied by an estimate of lifetime exposure (in µg/m^3) to estimate the lifetime cancer risk.

4.4.6. Previous IRIS Assessment: Inhalation Unit Risk

The previous IRIS assessment for hexavalent chromium was posted to the IRIS database in 1998. EPA's 1998 IRIS assessment classified Cr(VI) as "Group A—known human carcinogen by the inhalation route of exposure" under the 1986 classification guidelines. This was based on evidence of a causal relationship between inhalation of Cr(VI) and increased incidence of lung cancer in humans in occupational settings. An inhalation unit risk (IUR) for Cr(VI) of 1.2×10^{-2} per µg/m^3 was calculated based on increased incidence of lung cancer in chromate workers (Mancuso, 1997, 1975). Because Mancuso et al. (1997, 1975) only provided total chromium data, there was uncertainty in the 1998 IUR which led to an underestimation of risk.

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