OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds

Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Public Review Draft

January 2021

Air, Community, and Environmental Research Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Page Intentionally Left Blank

Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1
Public Review Draft

Prepared by the
Office of Environmental Health Hazard Assessment

Lauren Zeise, Ph.D., Director

Authors

Rona M. Silva, Ph.D.

Technical Reviewers

Daryn E. Dodge, Ph.D.

John D. Budroe, Ph.D.

David M. Siegel, Ph.D.

January 2021

Page Intentionally Left Blank

Table of Contents

List of Tables

Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Reference Exposure Levels

1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD; 2008) in response to this statutory requirement that describes methodology for deriving acute, chronic, and 8-hour Reference Exposure Levels (RELs). RELs are airborne concentrations of a chemical that are not anticipated to result in adverse noncancer health effects for specified exposure durations in the general population and sensitive subpopulations thereof. In particular, the methodology explicitly considers possible differential effects on the health of infants, children, and other sensitive subpopulations in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*).

The methods described in the TSD were used to develop the RELs for inorganic water-soluble trivalent chromium [Cr(III)] compounds presented in this document. Insolubility of a Cr(III) compound in water is defined in this document as having a water solubility of ≤100 mg/L at 20˚C (USP, 2015). Cr(III) compounds that have a water solubility of >100 mg/L at 20˚C are considered water-soluble. This definition of solubility is only applicable to the present document for regulatory purposes and does not apply to other OEHHA documents and programs. The RELs developed in the present document will be added to Appendix D of the TSD.

Inhalation exposure to Cr(III) has been shown to cause adverse respiratory effects in animals and humans including but not limited to 1) sensitization and induction of asthma with repeated exposure; 2) allergic asthma with coughing, wheezing, difficulty breathing; and decrements in lung function with short-term exposure; and 3) increased lung weights, alveolar inflammation, and decrements in macrophage function with long-term exposure. The level of exposure required to induce asthma in Cr(III)-sensitized individuals is unknown to OEHHA at this time. Though the RELs discussed herein are intended to reasonably protect the public from adverse health effects resulting from exposure to inorganic water-soluble Cr(III) compounds, they may not protect all individuals previously sensitized to these chemicals. As a public health protective measure, OEHHA developed the RELs using literature summarized and referenced

herein that encompasses the relevant, peer-reviewed, published original studies and governmental reports available for Cr(III) through August 2020 .

Because of the level of scientific information contained in this document, additional explanations of concepts and terms are provided. These explanations appear in the main text and sometimes in footnotes. Therefore, those using reading-assistive software should consider enabling pronunciation of punctuation and symbols, and listen for links to footnoted text.

1.1 Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Acute REL

Reference exposure level	2.5 μg/m^3 (0.0025 mg/m^3)
Critical effect(s)	Enzyme release in bronchoalveolar lavage fluid of hamsters consistent with tissue injury, combined with some pathologic evidence of airway damage
Hazard index target(s)	Respiratory system

1.2 Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Chronic REL

Reference exposure level	0.34 μg/m^3 (3.42 × 10^{-4} mg/m^3)
Critical effect(s)	Inflammation of nasal and pulmonary epithelium in rats
Hazard index target(s)	Respiratory system

1.3 Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds 8-Hour REL

Reference exposure level	0.68 μg/m^3 (6.83 × 10^{-4} mg/m^3)
Critical effect(s)	Inflammation of nasal and pulmonary epithelium in rats
Hazard index target(s)	Respiratory system

List of Abbreviations

AAS	Atomic absorption spectrometry
ABS	Artificial blood serum
ADME	Absorption, distribution, metabolism, and excretion
AIC	Akaike information criterion
ALP	Alkaline phosphatase
AP	Acid phosphatase
atm	Atmosphere (unit of pressure)
BALF	Bronchoalveolar lavage fluid
$BMCL_{1SD}$	The 95% lower confidence interval limit of the BMR response rate
$BMCL_{05}$	The 95% lower confidence interval limit at the 5% response rate
BMDS	Benchmark dose modelling software
BMR	Benchmark response; 1 SD from the control mean
BW	Body weight
°C	Degrees Celsius (unit of temperature)
CARB	California Air Resources Board
CAS	Chemical Abstracts Service
CI	Confidence interval
Cr	Chromium
^{51}Cr	Chromium-51 isotope
$CrCl_3$	Chromium (III) chloride
$CrCl_3 \times 6H_2O$	Chromium (III) chloride hexahydrate
Cr(III)	Trivalent chromium
CrO_4^{-2}	Chromate oxyanion
Cr_T	Total chromium
Cr_2O_3	Chromium (III)/chromic oxide
Cr(VI)	Hexavalent chromium
CTI	California Toxics Inventory
d_a	Aerodynamic diameter
DPM	Diesel particulate matter
DS	Delayed-sacrifice
DSB	Double-strand break
ELISA	Enzyme-linked immunosorbent assay
ET-AAS	Electrothermal atomic absorption spectrometry
$FeCr_2O_4$	Chromite ore
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GD	Gestation day
GI	Gastrointestinal
Glu-6P-DH	Glucose-6-phosphate dehydrogenase
GSD	Geometric standard deviation
GTF	Glucose tolerance factor
HEC	Human equivalent concentration
HEPA	High-efficiency particulate air (filtration)
Hg	Mercury
HMWCr	High molecular weight Cr-binding substance
H_2O_2	Hydrogen peroxide
ICP-MS	Inductively coupled plasma mass spectrometry
Ig	Immunoglobulin
IS	Immediately sacrificed
K	Kelvin (unit of temperature)
K_{ow}	N-Octanol/water partition coefficient
$K_2Cr_2O_7$	Potassium dichromate
LDH	Lactate dehydrogenase
LMWCr	Low molecular weight Cr-binding substance
LOAEL	Lowest observed adverse effect level
$LOAEL_{HEC}$	Human-equivalent LOAEL concentration
LOD	Limit of detection
LOQ	Limit of quantification
MCE	Mixed cellulose ester
MMAD	Mass median aerodynamic diameter
Mn	Manganese

List of Abbreviations (continued)

mol	Moles (# of particles in a substance)
MPPD	Multiple-Path Particle Dosimetry Model
MV	Minute volume
MV_A	Minute volume for animal
MV_H	Minute volume for human
NA	Not available
NaCl	Sodium chloride
Na_3CrO_2	Sodium chromite
NACDG	North American Contact Dermatitis Group
NBT	Nitroblue tetrazolium
NOAEL	No observed adverse effect level
NO_2	Nitrogen dioxide
NO_x	Oxides of nitrogen
NT	Not tested
NTP	National Toxicology Program
Ni	Nickel
OH^-	Hydroxide ion
$^*OH$	Hydroxyl radical
O_3	Ozone
$^*O_2^-$	Superoxide ion
OEHHA	Office of Environmental Health Hazard Assessment
OSHA	Occupational Safety and Health Administration
PBPK	Physiologically-based pharmacokinetic (model)
PC_{20}	Provocation concentration [of methacholine] causing a 20% decrease in FEV1
PE	Post exposure
PEL	Permissible exposure limit
PEFR	Peak expiratory flow rate
PFT	Pulmonary function test
PM	Particulate matter
PM_{10}	Particulate matter ≤10 µm in aerodynamic diameter
POD	Point of departure
PO_4^{-3}	Phosphate oxyanion
PS	Post sensitization
RBC	Red blood cell
REL	Reference Exposure Level
RDDR	Regional deposited dose ratio
RH	Relative humidity
ROS	Reactive oxygen species
SCI	Subcutaneous injection
SIDMS	Speciated Isotopically Dilution Mass Spectrometry
SOA	Secondary organic aerosol
SO_4^{-2}	Sulfate oxyanion
SO_2	Sulfur dioxide
T	Temperature
TB-ADJ	Terminal bronchiole-alveolar duct junction
Tf	Transferrin
TSD	Technical Support Document
TWA	Time-weighted average
$t_{1/2\text{-}A}$	Atmospheric half-life
$t_{1/2\text{-}U}$	Time needed for half of the inhaled Cr dose to be eliminated via urine
UF	Uncertainty factor
$UF_{A\text{-}d}$	Toxicodynamic portion of the interspecies uncertainty factor
$UF_{A\text{-}k}$	Toxicokinetic portion of the interspecies uncertainty factor
$UF_{H\text{-}d}$	Toxicodynamic portion of the intraspecies uncertainty factor
$UF_{H\text{-}k}$	Toxicokinetic portion of the intraspecies uncertainty factor
UF_L	LOAEL uncertainty factor
US EPA	United States Environmental Protection Agency
WB	Whole body
WBC	White blood cell; leukocyte
µCi	Microcurie

2. Physical & Chemical Properties

Table 1a. Cr(III) ion and selected soluble[b] trivalent chromium compounds.

Molecular Formula	**Cr^{3+}**	**$Cr(NO_3)_3$**	**$Cr_2(SO_4)_3 \times x(H_2O)$**	**$Cr_2(OH)_x(SO_4)_y$ $NaSO_4$ $2H_2O$**
Synonyms	Chromium (III), chromic ion; chromium (III) ion; chromium (3^+)	Chromic nitrate, chromium (III) nitrate, chromium trinitrate	Chromium (III) sulfate hydrate	Basic chromium (III) sulfate, chromium hydroxide sulfate, basic chromic sulfate, Chromedol, Peachrome
Chemical Abstracts Service (CAS) Number	16065-83-1	13548-38-4	Variable	Variable
Molecular Weight (g/mol)	51.996	238.01	>392.16	Variable
% Cr[a]	100	22	Variable	Variable
Water Solubility (g/L H_2O at 20°C)	NA	"Very good"[b]	"Soluble"[b]	"Soluble"[b]
Reference	NCBI (2019a)	NCBI (2019b); Hammond (2011)	NCBI (2019e)	Derelanko *et al*. (1999)

Abbreviations: NA – not available

[(a)] % Cr = (molecular weight Cr) × (mol Cr per mol of stated species) ÷ (molecular weight species) × 100

[(b)] In some cases, exact measures of water solubility were not found by OEHHA, but qualitative descriptions were. In these cases, the descriptions were included in quotations. However, these descriptions may not coincide with OEHHA's definition (>100 mg/L, or >0.1 g/L, at 20˚C; USP, 2015) of water solubility.

Table 1a. Selected soluble[b] trivalent chromium compounds (continued).

Molecular Formula	**$Cr_4(SO_4)_5(OH)_2$**	**$Cr(HO_4S)_3$**	**$Cr(SO_4)(OH)$**	**$CrCl_3 \times 6H_2O$**
Synonyms	Basic chromium (III) sulfate, chromium hydroxide sulfate, basic chromic sulfate, Chromedol, Peachrome	Same as previous	Same as previous	Chromium (III) chloride hexahydrate, chromic chloride hexahydrate
Chemical Abstracts Service (CAS) Number	39380-78-4	39380-78-4	12336-95-7	10060-12-5
Molecular Weight (g/mol)	722.31	343.21	165.07	266.436
% Cr[a]	29	15	31	20
Water Solubility (g/L H_2O at 20°C)	"Soluble"[b]	Soluble (assumed)[c]	2×10^3	590
Reference	Sigma-Aldrich (2017); LOBA Chemie (2014)	NCBI (2019f)	NCBI (2019d)	NCBI (2019c)

Abbreviations: NA – not available

(a) % Cr = (molecular weight Cr) × (mol Cr per mol of stated species) ÷ (molecular weight species) × 100

(b) In some cases, exact measures of water solubility were not found by OEHHA, but qualitative descriptions were. In these cases, the descriptions were included. However, these descriptions may not coincide with OEHHA's definition (>100 mg/L, or >0.1 g/L, at 20˚C; USP, 2015) of water solubility.

(c) Solubility assumed by OEHHA based upon similarity to other chemicals with the same name and/or CAS number.

Table 1b. Selected insoluble[b] trivalent chromium compounds.

Molecular Formula	$CrCl_3$	$Cr_2(SO_4)_3$	Cr_2O_3
Synonyms	Chromium (III) chloride, trichlorochromium, chromic chloride anhydrous, chromic (III) chloride, chromium (3^+) chloride	Anhydrous chromium (III) sulfate	Chromium (III) oxide, chromic oxide, dichromium trioxide
Chemical Abstracts Service (CAS) Number	10025-73-7	10101-53-8 and others	1308-38-9
Molecular Weight (g/mol)	158.35	392.16	151.99
% Cr[a]	33	26.5	68
Water Solubility (g/L H_2O at 20°C)	"Insoluble"[b]	"Insoluble"[b]	3.13×10^{-6} (pH=6); 2.96×10^{-6} (pH=8)
Reference	NCBI (2020b)	NCBI (2019e)	NCBI (2020a)

Abbreviations: NA – not available

(a) % Cr = (molecular weight Cr) × (mol Cr per mol of stated species) ÷ (molecular weight species) × 100

(b) In some cases, exact measures of water solubility were not found by OEHHA, but qualitative descriptions were. In these cases, the descriptions were included. However, these descriptions may not coincide with OEHHA's definition (>100 mg/L, or >0.1 g/L, at 20˚C; USP, 2015) of water solubility.

3. Production, Major Uses, Measurement, and Occurrence

Chromium (Cr), one of the most common elements in the earth's crust and sea water, is a naturally occurring heavy metal that can exist in oxidation states ranging from $^{-}2$ to $^{+}6$ (Shupack, 1991). Metallic and hexavalent Cr [Cr(0) and Cr(VI), respectively], for example, are commonly produced by industrial processes. Cr(VI) occurs rarely in nature without anthropogenic interference (Sun *et al.*, 2015). Cr(III) is generally the most thermodynamically stable state of Cr, and most stable Cr compounds exhibit the Cr^{+3} oxidation state. It should be noted that Cr(III) can be oxidized to form Cr(VI), e.g. at high temperatures with atmospheric oxygen during wildfires, but Cr(III) is still the most prevalent state in the environment (IPCS, 2009). Except for acetate, nitrate, sulfate, and chloride-hexahydrate salts, Cr(III) compounds are often insoluble in water (ATSDR, 2012).

3.1 Production

Production of atmospheric Cr(III) can occur with 1) mining of chromite ore ($FeCr_2O_4$), an iron Cr(III) oxide; 2) processing of $FeCr_2O_4$ into sodium chromate and dichromate, both Cr(VI) chemicals; and 3) refinement of $FeCr_2O_4$ into ferrochromium alloys and Cr (0) metal. Additional refinement commodities include Cr(III) oxide (Cr_2O_3)-based refractory products like bricks and sands for high temperature applications. Though California was historically one of the few states authorized by the federal government for $FeCr_2O_4$ mining, the practice was only economically feasible domestically during times of political conflict, so the United States has imported all its chromite since 1961 (OHS, 2018).

Atmospheric Cr(III) is also produced through the conversion of airborne Cr(VI). According to the US Environmental Protection Agency (US EPA, 1998), airborne Cr(VI) eventually reacts with dust particles or other pollutants to form Cr(III). Reduction of Cr(VI) to Cr(III) has occurred through the action of vanadium (V^{2+}, V^{3+}, and VO^{2+}), iron (Fe^{2+}), and arsenic (As^{3+}) cations, and hydrogen sulfite anions (HSO^{3-}), with the estimated Cr(VI) atmospheric half-life in the range of 16 hours to 5 days (ATSDR, 2012). In this case, the atmospheric half-life ($t_{1/2\text{-}A}$) of Cr(VI) is the time it takes for half of the emitted Cr(VI) to be converted to Cr(III). Cr is generally removed from the air by atmospheric fallout (settling to the ground) or precipitation (e.g. rain). However, the removal time is dependent upon the particle size and density, such that smaller lighter particles remain aloft for a longer duration relative to larger heavier ones (US EPA, 1998).

Other potential sources of atmospheric Cr(III) emissions in California include industrial plants producing Cr(III) refractory materials or cement, automobile catalytic converters, and leather-tanning and metal-plating facilities.

3.2 Major Uses

Cr(III) compounds are used as dietary supplements, pigments, catalysts, leather tanning agents, and decorative plating media.

3.2.1 Cr(III) in Leather Tanning Operations

In the “wet blue” Cr(III) tanning process, “unhaired” animal hides undergo multiple rounds of acidification and basification to permanently alter the hide, make it more durable and less susceptible to decomposition, and transform it into a finished product. During tanning steps, a Cr(III) salt is added to animal hides previously pickled in acidic media. Addition of Cr(III) to acidified hides allows it to fit between collagen fibers in the hide. Subsequent basification of the media with sodium bicarbonate to an approximate pH = 4 induces cross-linking between the Cr and collagen (FAO, 1996).

The type of Cr(III) added in tanning/re-tanning steps is variable but has been reported by the Danish EPA (2012) as primarily Cr(III) hydroxide sulfate, i.e. $Cr(SO_4)(OH)$. However, Cr(III) potassium bisulfate, i.e. $KCr(SO_4)_2$, and violet Cr(III) acetate $[Cr(H_2O)_6](CH_3COO)_3$ have also been reported for use in specialty applications (Danish EPA, 2012).

Animal hides are left in the alkaline Cr solutions for 24-48 hours to remove water molecules bound to collagen in the skin, and create a thinner, softer leather than can be obtained via vegetable tanning. After soaking, the wet hides are fed into a press that removes most of the tanning liquid, processed further, and buffed as part of a finishing procedure. Cr exposures occur most during preparation of the tanning solution, pressing, or buffing via inhalation of or dermal-to-oral contact with powdered Cr(III) salts, tanning solution, or buffing-related particulates (US EPA, 1995).

Cr(VI) is not added directly but may be formed via oxidation of Cr(III) due to factors including but not limited to pH, temperature, UV light, or unsuitable hide-storage conditions (Basaran *et al.*, 2008). Generally, studies into leather-related Cr(VI) formation have focused on Cr(VI) content in finished leathers, not the tanning media. Therefore, it is unclear to OEHHA exactly when Cr(VI) is most likely to be formed. However, at least one report suggests oxidation may occur after tanning, during acid-neutralization or dyeing processes, when the media pH is high (Danish EPA, 2012).

3.2.2 Cr(III) in Chrome-Plating Processes

Cr(III) plating involves the use of electrical currents to reduce dissolved Cr(III) to Cr (0), which then deposits on the item(s) to be plated. These processes take place in large bath tanks and result in aerosolization of water and Cr(III) and/or Cr(VI) in a mist. Specifically, generated gas bubbles rise to the surface of the tank and burst out of the bath as tiny droplets. These Cr emissions are regulated by federal and state agencies (US EPA, 2010; CARB, 2018) and generally controlled through the use of mist/fume suppressants and wet scrubbers. The former decrease the surface tension of the Cr bath solution to prevent entrainment of solution droplets in ambient air, and the latter remove airborne pollutants from industrial exhaust streams.

At the time of the present report, there were only five registered Cr(III) plating facilities in California. However, according to an analysis by the California State Assembly (2005), metal-plating facilities in California are generally small businesses in communities of color, in close proximity to sensitive receptors (e.g., schools and hospitals). In their *Airborne Toxic Control Measure for Chromium Plating and Chromic Acid Anodizing Facilities*, The California Air Resources Board (CARB) requires total Cr (Cr_T) emissions from Cr(III) plating facilities to be controlled by one of two methods. In Method 1, add-on air pollution control equipment or chemical/mechanical fume suppressants can be used to ensure Cr_T emission levels are ≤ 0.01 mg/dry standard cubic meter (dscm; a value adjusted for moisture content). In Method 2, a chemical fume suppressant containing a wetting agent can be added as a bath ingredient, and the owner/operator of the facility agrees to comply with certain recordkeeping and reporting provisions detailed in the regulation. Method 2 is generally more commonly used since wetting agents are part of the plating chemistry and less expensive than add-on controls.

Cr(III) has been used as an alternative to the Cr(VI)-based chrome-plating processes prevalent in the industry. Cr(III) plating processes are typically recognized as more energy-efficient than those using Cr(VI). Because Cr(III) sulfates or Cr(III) chlorides are the primary chemicals used in Cr(III) plating bath media, Cr(III) plating processes are also less likely to produce environmental and health concerns on par with Cr(VI). However, Cr(III) plating processes are also less widely used due to greater chemical costs, inferior corrosion resistance, differences in coating color, and the need for more precise parameter (e.g. temperature, pH) controls relative to Cr(VI) ones (FTI, 2003).

Experimental Cr(III) plating solutions have been reported to contain chromic chloride [$CrCl_3$; (Song and Chin, 2002)]; chromic chloride hexahydrate [$CrCl_3 \times 6H_2O$; (Baral and Engelken, 2005; Suarez *et al.*, 2012)]; Cr(III) potassium sulfate dodecahydrate [$KCr(SO_4)_2 \times 12H_2O$; (Protsenko *et al.*, 2014)]; basic Cr (III) as $Cr_2(SO_4)_3 \times 6H_2O$ (Edigaryan *et al.*, 2002), or $Cr_2(SO_4)_n(OH)_{6-2n}$, where n<3 (Kwon SC, 2012; Protsenko

and Danilov, 2014). Other added chemicals include but are not limited to complexing agents like formate, and buffers such as boric acid.

3.3 Measurement of Airborne Cr

Measurements of airborne Cr are complicated by the need to minimize unwanted redox reactions that lead to Cr(III) ↔ Cr(VI) species interconversions. Basic (pH > 7) filters have been used as collection media in attempts to mitigate these conversions. However, this sampling method has not proven reliable. Factors that affect Cr conversions during sampling are discussed below in the summary of a study by Huang *et al*. (2013).

Controlled chamber and outdoor field experiments by Huang *et al*. (2013) revealed:

1) ambient sulfur dioxide (SO_2) can reduce Cr(VI) to Cr(III) on filters laden with diesel particulate matter (DPM) or secondary organic aerosols (SOAs), i.e. aerosols produced through the oxidative interactions of sunlight, volatile organic compounds, and other airborne chemicals;

2) DPM and SOA are separately capable of reducing Cr(VI) to Cr(III) in a clean-air environment removed of particulate matter (PM), organics, oxides of nitrogen (NO_x), ozone (O_3), and SO_2; and

3) in the presence of stable reactive oxygen species (ROS), SOA is sufficient to oxidize Cr(III) to Cr(VI), and this oxidation can increase (i.e. more conversion can occur) as relative humidity (RH) and ROS levels increase.

In the 2013 report by Huang *et al*., oxidized organic compounds in DPM and SOA were said to enhance the ability of airborne PM to attract and hold water from the surrounding environment, and this enhanced PM hygroscopicity facilitated Cr(VI) reduction. Concurrent oxidation by SOA was suggested to be due to stable ROS, e.g. organic peroxides and hydroperoxides, present in the SOA since ROS constitute approximately 47-85% of SOA mass. The authors cited two supporting studies (Nico *et al.*, 2009; Torkmahalleh *et al.*, 2013) reporting competing Cr redox reactions using different PM compositions and environmental conditions, and stated that atmospheric SOA could affect Cr during sampling, thus necessitating the simultaneous measurement of Cr(VI) reduction and Cr(III) oxidation using a method such as Speciated Isotopically Dilution Mass Spectrometry (SIDMS).

In their study of redox reactions with mixed metals including manganese (Mn), Cr, and Fe, Nico *et al*. (2009) suggested that Mn in ultrafine PM drove the oxidation of Cr(III) to

Cr(VI). Laboratory experiments by Torkmahalleh *et al*. (2013) attempted to establish the role of O_3 and particle-bound ROS on Cr speciation. Both O_3 and ROS were shown to participate in competing redox reactions, increasing the oxidation of filter-bound Cr(III) to Cr(VI) and the reduction of Cr(VI) to Cr(III) relative to control conditions without O_3 and/or ROS. Oxidation by O_3 slowed with decreased temperatures (12°C versus 24°C), suggesting that Cr(III)-to-Cr(VI) conversions could be limited at lower temperatures. Overall, results suggested to Torkmahalleh *et al*. (2013) that in the presence of oxidants and reductants, ambient Cr would not be completely converted to Cr(III) or Cr(VI) but rather that the ratio of the two species would be controlled by environmental conditions (e.g. temperature, RH) that affect steady state.

This was supported in the study by Huang *et al*. (2013), where seasonal variation was also shown to play a role in Cr interconversions, with Cr(VI) reduction occurring in summer and winter sampling events irrespective of whether basic filter media was used. According to the authors, the reduction occurred more in summer versus winter likely due to higher temperatures leading to faster chemical reactions, atmospheric water vapor resulting in aqueous-phase Cr reactions, and increased photochemical activities producing elevated O_3 and other oxidants in the atmosphere during summer. They recommended in-situ monitoring of Cr(VI) reduction and the use of the US EPA method 6800 to improve accuracy of Cr(VI) measurements.

US EPA's Method 6800 (2014) employs a two-step approach using isotope dilution mass spectrometry (IDMS) to determine total concentrations of elements and molecules and SIDMS to quantify elemental and molecular species (i.e. those that differ in isotopic composition, oxidation or electronic state, or in the nature of their complexed or covalently bound substituents). Concentrations can be quantified at the parts per billion, parts per trillion, and sub-parts per trillion levels in various types of samples including but not limited to bodily fluids, solids, and water (US EPA, 2014). Given that numerous ambient factors have been shown to have redox effects on Cr, the accuracy of future assessments of airborne Cr(III) could be improved by employing methodology such as that described in Method 6800 versus simply using basic filter media.

3.4 Occurrence

3.4.1 Outdoor Emissions of Cr(III)

Cr(III)-specific emissions information was not available for California. The most recent finalized modeled estimates of total Cr emissions from CARB's Statewide 2008 California Toxics Inventory (CTI) were 19 tons from aggregated stationary sources, 9 tons from on-road mobile sources, and 114 tons from area-wide sources. Stationary sources include point sources such as smelters and foundries. Mobile sources consist of on-road vehicles like passenger cars, motorcycles, buses, and light- and heavy-duty trucks. Area-wide sources are spread over large areas but do not have specific point locations. Some examples of area-wide sources include consumer products, unpaved roads, and soil- or road-dust resuspension. The most recently posted (2010) draft CTI showed that Cr emissions were approximately 10, 21, and 108 tons from aggregated stationary, on-road mobile, and area-wide sources, respectively, suggesting an approximate ±10-ton difference from the 2008 stationary and on-road mobile source emissions. According to CARB (G. Ruiz personal communication, May 28, 2018), though the values reported above were not generally meant to include Cr(VI) emissions, it is possible that Cr(VI) emissions were included as part of undifferentiated total chromium measurements/estimates used by CARB in generating the 2008 and draft 2010 CTIs.

Publicly available reports of Cr(III) emissions are limited primarily because governmental regulatory and public interests are widely focused on Cr(VI). Though measured industrial Cr(III) emissions from California facilities could not be found, OEHHA located one study by US EPA (1992) that reported Cr(III) emissions from a chrome-plating facility in Seneca, South Carolina during the week of June 8, 1992.

US EPA (1992)

According to the study authors, the facility operated several cleaning/rinsing tanks and five metal-plating tanks using a Cr(III) plating process in the production of metal shafts for golf clubs. The facility was chosen for emissions testing because of the Cr(III) plating process employed and the presence of an exhaust hood that was well-suited for sampling emissions. The report did not state which specific chemicals were being used in the plating tanks, but they were said to hold 5400 gallons (20,400 L) of plating solution at Cr(III) concentrations ranging 2.8 - 3.2 oz/gallon (21 – 24 g/L).

In the US EPA (1992) study, three 3-hour air sampling runs were performed using a modified version of US EPA Method 13B (1980) under isokinetic (constant velocity) conditions. Although Method 13B was designed for determination of total fluoride

emissions from stationary sources, in this study, Cr_T and Cr(VI) masses were measured and used to calculate that of Cr(III). Isokinetic sampling is widely used in particle measurements from ambient air, power plants, and scrubbers. The scrubber at the facility was not in use. However, a wetting agent (Regulator™) was added to the plating tank solution to suppress Cr(III) emissions. Additions were done manually at the start of a run, and automatically via a controller based upon the amount of current supplied to the plating tank. The wetting agent was supposed to reduce the surface tension of the plating bath solution from approximately 72 dynes/cm to < 40 dynes/cm to provide more uniform plate thickness over the surface of the golf club shafts, and decrease emissions from the bath. No information was provided regarding the provenance or contents of the Regulator™ product, and OEHHA was unable to locate this information.

In general, air samples were collected, from a straight section of duct work between the scrubber and the point at which the exhaust duct intersected the roof, using a glass impinger sampling train[1]. Sample train, reagent, and field blank controls were included but not described. These are typically included as quality controls to test for potential contamination introduced by the sampling equipment, sampling media, and sample handling, respectively. Two test ports were cut into the duct-work at 90° angles from each other, and according to the authors of the study, 12 points were sampled at each of the two ports, for a total of 24 sample points. It is unclear to OEHHA whether all 24 points were sampled during each run. Sampling occurred when the plating tank solution was homogenously mixed with Regulator™, and other plating process conditions were within normal ranges for the facility.

During each of the air sampling runs, surface tension measurements were made and grab samples were taken of the plating bath solution. During Run #1, and after the manual addition of Regulator™ at the beginning of Run #2, it was noted that surface tension was still above 40 dynes/cm. Laboratory testing was done to determine the effect of Regulator™ on the plating solution. In these lab tests, a sample of the latter was spiked with varying unspecified amounts of Regulator™, and surface tension was measured with a stalagmometer[2]. Results indicated that further addition of Regulator™

[1] Impingers are specially designed tubes used for collecting airborne chemicals into a liquid medium. In the case of the US EPA (1992) study, the medium was sodium hydroxide. With impinger sampling, a known volume of air is bubbled through the impinger(s) containing the medium, which will chemically react with or physically dissolve the chemical of interest (SKC, 1996), thus trapping it for future recovery and analysis.

[2] A stalagmometer, also known as a stactometer or stalogometer, is a glass capillary tube with a widened midsection and a narrowed tip that forces fluid in the tube to exit as a drop when the tube is held vertically. By measuring the weight of fallen drops of a fluid of interest, surface tension can be calculated

to the facility plating tank would not significantly reduce the surface tension of the bath, so manual additions were not made for Run #3.

After each test run, air and plating solution samples were recovered immediately and stored in a cooler during transport prior to analysis of Cr_T and Cr(VI) in air, and Cr_T in the plating bath. Cr_T levels were determined by inductively coupled plasma (ICP) spectrometry; Cr(VI) was measured by ion-chromatography with a post column reactor; and ambient Cr(III) concentrations were calculated by subtracting Cr(VI) content from Cr_T in air.

Results showed some between-run variability in air samples, but average mass emissions consisted of approximately 87% Cr(III) and 13% Cr(VI). Cr determinations from air are shown in Table 2 below.

using the equation $mg = 2\pi r\sigma$, where mg is the weight of a drop of fluid, $\pi = 3.14$, r is the radius of the capillary tube, and σ is the surface tension.

Table 2. Analytical results of chromium (Cr) mass emission testing at a Cr(III) plating facility in Seneca, South Carolina.

Endpoint	Cr Species	Sampling Run #1	Sampling Run #2	Sampling Run #3	Average
Total Mass Collected (µg; % of total)	Cr_T	36.90	156.00	61.10	84.67
	Cr(VI)	10.20; 28%	14.90; 10%	8.01; 13%	11.04; 13%
	Cr(III)[a]	26.70; 72%	141.10; 90%	53.09; 87%	73.63; 87%
Emission Concentration (mg/dscm)	Cr_T	1.29×10^{-2}	4.78×10^{-2}	1.91×10^{-2}	2.66×10^{-2}
	Cr(VI)	3.6×10^{-3}	4.6×10^{-3}	2.5×10^{-3}	3.6×10^{-3}
	Cr(III)[a]	9.3×10^{-3}	4.32×10^{-2}	1.66×10^{-2}	2.30×10^{-2}
Mass Emission Rate (mg/hr)	Cr_T	192.3	845	334.7	457.3
	Cr(VI)	53.16	80.74	43.88	59.25
	Cr(III)[a]	139.2	764.3	290.8	398.1

Table modified from US EPA (1992) Table 3.2. Abbreviations: Cr(III) – trivalent chromium; Cr_T – total chromium; Cr(VI) – hexavalent chromium; dscm – dry standard cubic meter (value adjusted for moisture content).

[(a)] US EPA values calculated by subtracting Cr(VI) measurements from those of Cr_T.

No reasons were given to explain the presence of Cr(VI) or between-run variability in Cr air concentrations, and these were not obviously correlated to specific sampling or stack conditions. Sample train and reagent blank levels of Cr_T were below the limits of detection (<0.62 µg and <0.736 µg, respectively) suggesting a low likelihood of contamination from the sampling apparatus. Cr_T concentrations in the plating bath solution ranged from 18,850 µg/mL (18.85 mg/mL) in Run #1 to 18,100 µg/mL (18.1 mg/mL) in Runs #2 and 3 — a 4% difference — indicating that the variability in Cr

air samples could not be due to Cr bath concentrations alone. Measured bath operating parameters like amperes (range = 5300 – 5600), voltage (range = 10.6 – 10.8 volts), and plating solution temperature (range = 97 – 98 °F) were fairly constant with a maximum percent difference of approximately 6%, 2%, and 1%, respectively, between runs. Bath pH was not reported. Average surface tension of the plating solution, which was collected prior to and at the midpoint and end of each run, ranged from 43-53 dynes/cm (average = 48 dynes/cm). This was a 21% difference; however, surface tension was highest in Runs #2 and 3 when air Cr_T concentrations were highest. No measurements were taken without the addition of Regulator™, so its influence on emissions was unclear to the authors of the study and OEHHA. Other conditions that may have contributed to variability in measured concentrations of Cr include, but are not limited to, stack temperature, moisture, air flow velocity, and instability of Cr(VI) during sample storage. Post collection sample loss is possible but was not mentioned. Without additional information regarding ambient air quality during sampling (e.g. PM concentration and composition) and the chemical composition of the plating bath and Regulator™ solutions, it is difficult for OEHHA to assuredly determine whether Cr(VI) emissions may have resulted from the Cr(III) plating operations in the Seneca facility.

Given the reducing conditions in Cr plating baths in general, it may seem unlikely that a Cr(III) bath solution unmodified by other metals or chemical additives would contain Cr(VI). However, coating bath solutions are complex and variable, often composed of proprietary chemical mixtures. Previous studies indicate Cr(VI) can be formed with Cr(III) coating processes (Protsenko, 2014; Hesamedini and Bund, 2017). Additional studies are needed to fully and accurately assess the emissions associated with present-day Cr(III) plating facilities and risks thereof.

3.4.2 Measured Occupational Exposures to and Indoor Concentrations of Cr(III)

Cr(III) exposure occurs primarily through diet (including supplements), inhalation, or direct contact with chrome-tanned leather, Cr(III)-containing cosmetics, stainless steel items, prosthetic implants, or orthodontic appliances (WHO, 2009). The average intake of Cr via inhalation has been estimated at <0.2 – 0.6 µg per day (ATSDR, 2012). Though publicly available, peer-reviewed human Cr(III) exposure studies are limited and focused on occupational exposures, those found by OEHHA are discussed below. Studies with mixed metal or mixed Cr [Cr(III) and Cr(VI)] exposures were generally not included.

Kiilunen et al. (1983)

Occupational exposure and urinary excretion of Cr was measured in workers exposed to Cr(III) in a Cr lignosulfonate manufacturing facility. Urinary excretion of Cr is discussed in Sections 4.4, 4.6, and 4.7.

Lignin is a complex organic polymer found in the cell walls of rigid, woody plants. Lignosulfonates are water-soluble polyanionic lignin polymers. Cr lignosulfonate is used as a conditioner in oil drilling (Chen *et al.*, 2018). Though dichromate (a hexavalent compound) is used to make Cr lignosulfonate, the former is ultimately reduced to Cr(III) during the lignosulfonate production process. Five workers from the packing department of the factory participated in the study, and three of them used masks. No other subject information was provided except that all five were said to be exposed only to the final Cr(III) product, not the dichromate component used in its manufacturing.

Personal (breathing zone) and stationary (control room and packing area) dust samples were collected on cellulose ester membrane filters over two 4-hour work periods for three consecutive days. Total dust was gravimetrically measured, dust morphology was observed by scanning electron microscopy, and Cr_T was quantified using atomic absorption spectrophotometry[3] (AAS) with an air-acetylene flame. Cr valence was determined in aqueous solutions and dry dust samples of the Cr lignosulfonate product by the diphenyl carbazide color reaction, a method that allows quantification of Cr(VI), and x-ray photoelectron spectroscopy, a method that measures elemental composition.

Total dust levels ranged from 100 – 12,000 µg/m^3 (0.1 – 12 mg/m^3) in personal samples and 7000 – 41,000 µg/m^3 (7 – 41 mg/m^3) in stationary samples over the three collection days. About 30% of dust particles were <5 µm in diameter. Dust samples contained an average of 2% Cr_T (range = 1 – 4.2%) in comparison to the finished Cr lignosulfonate product which was composed of 6% Cr_T. All Cr in the dust samples was Cr(III). Personal Cr_T from air samples was highly variable among the different subjects. Levels for the group ranged from 2 – 230 µg/m^3 (0.002 – 0.230 mg/m^3), and individual averages ranged from 11 – 80 µg/m^3 (0.011 – 0.08 mg/m^3). As a point of comparison, personal Cr_T exposures were less than the current California Occupational Safety and Health Administration (CAL/OSHA) permissible exposure limit (PEL).

[3] Atomic absorption spectrophotometry uses the absorption of light by free metallic atoms in the gaseous state to quantify chemicals in liquid or solid samples. In this process, the sample is dried, vaporized, and atomized to enable quantification of metal elements. Atomizers are variable, and commonly used types include but are not limited to flame (e.g. air-acetylene) and electrothermal atomizers.

The PEL is a maximally permitted 8-hour time-weighted average (TWA)[4] concentration of 500 µg/m^3 (0.5 mg/m^3) for airborne Cr(III) compounds (8 CCR, GISO, §5155, Table AC-1, 1976).

Aitio et al. (1984)

In their investigation of occupational exposure to Cr, Aitio *et al.* (1984) took personal and stationary air samples in a Finnish leather tanning facility that was using a Cr(III) "wet-blue" process, and assessed the results in relation to levels of Cr in urine and blood of tannery workers performing different tasks. Results of biological assessments are discussed in Section 4.6, herein.

In the study by Aitio *et al*. (1984), leather hides were being treated overnight in large rotating tanning drums containing Cr(III) sulfate, a water-soluble Cr(III) chemical. No chemical-specific information (e.g. CAS number, chemical formula, purity) was provided regarding this tanning liquid. Two male smokers who fed Cr-soaked hides into a press, and four individuals who stood on the other side of the press and received the hides comprised the study population. The former are referred to herein as "feeders;" the latter are referred to as "receivers." Sex and smoking statuses of the receivers were not stated by Aitio *et al*. Personal and stationary air samples were collected for six hours onto ester membrane filters using a monitor with a ≤4-mm (4000-µm) size restriction. Filters were analyzed gravimetrically for dust mass and subsequently dissolved in nitric acid for quantification of Cr_T via graphite furnace (electrothermal) atomic absorption spectrometry (ET-AAS). It is unclear to OEHHA whether air samples were collected on more than one workday. Limits of detection and quantification (LODs and LOQs, respectively) and other potential sources of error were generally not reported for the various measurements.

TWA Cr_T exposure concentrations in the Finnish leather tanning facility reported by Aitio *et al*. (1984) were much lower than the current Cal/OSHA PEL. Task-driven differences were indicated by approximately 2-fold greater breathing zone dust and 6-fold greater breathing zone Cr_T in hide-feeders versus –receivers. Measured dust concentrations ranged from 100 – 1300 µg/m^3 (mean = 700 µg/m^3) for feeders and 100 – 600 µg/m^3 (mean = 300 µg/m^3) for receivers. These values equate to 0.1 – 1.3 mg/m^3 (mean = 0.7 mg/m^3) and 0.1 – 0.6 mg/m^3 (mean = 0.3 mg/m^3), respectively. Cr_T

[4] When the air sampling duration is "T" and the measured concentration of a specific chemical is "C", the TWA is calculated by adding the T × C product for each sampling period and dividing the answer by the sum of all T's. For example, if occupational air sampling occurred over two sampling periods (T_1 and T_2), where T_1 was 3 hours and T_2 was 5 hours, and resulting exposure concentrations (C_1 and C_2) were measured at 7 mg/m^3 and 10 mg/m^3, respectively, the 8-hour TWA would be calculated as follows: TWA = [($T_1 \times C_1$) + ($T_2 \times C_2$)] ÷ ($T_1 + T_2$) = [(3 × 7) + (5 × 10)] ÷ (3 + 5) = [21 + 50] ÷ 8 ≈ 8.9 mg/m^3.

measured at 4 – 29 µg/m^3 (mean = 13 µg/m^3) for feeders and 1 – 3 µg/m^3 (mean = 2 µg/m^3) for receivers. The levels correspond to 0.004 – 0.029 mg/m^3 (mean = 0.013 mg/m^3) and 0.001 – 0.003 mg/m^3 (mean = 0.002 mg/m^3), respectively. Personal dust and Cr_T exposures in receivers were similar to levels measured by stationary samplers. Because their technique for sampling respirable particles (i.e. particulate matter ≤10 µm in aerodynamic diameter[5]; PM_{10}) excluded large droplets which may be absorbed from the GI tract upon hand-to-mouth exposure, Aitio *et al*. (1984) stated that their air sampling procedure was "misleading." More precisely, the methods did not allow for apportionment of effects resulting from oral exposure.

Cavalleri and Minoia (1985)

Cavalleri and Minoia determined Cr_T, Cr(VI), and Cr(III) in personal air samples, urine, and blood of three groups of workers. However, their materials and methods were minimally described. Their experiments with biological samples are discussed in Section 4.6 of the present document.

Personal air samples were collected from a total of 79 workers. Of these subjects, 42 (Group A) were exposed to Cr(III) and Cr(VI) during electrode welding operations, 15 (Group B) were exposed mainly to $Cr_2(SO_4)_3$, and 22 (Group C) were exposed mainly to Cr(VI) via water-soluble $K_2Cr_2O_7$ (potassium dichromate) PM and chromic acid fumes and PM. The occupations of and tasks performed by Group B and Group C workers were not stated, and 8-hour TWA Cr_T exposures were much higher than those reported by Aitio *et al*. (1984) ranging from 18 to 1700 µg/m^3 (0.018 to 1.7 mg/m^3) for all groups. Associated Cr(III) concentrations for Groups A-C ranged from 5 to 1690 µg/m^3 (0.005 to 1.69 mg/m^3) accounting for approximately 20-25% of Cr_T in Group A, nearly 100% in Group B, and 30-55% in Group C.

Randall and Gibson (1987)

Similar to Aitio *et al*. (1984), Randall and Gibson measured serum and/or urine Cr levels of tannery workers to determine whether those biological indices could be correlated to inhalation exposure. Experiments performed on the biological samples are discussed in Section 4.6 of the present document.

Four different tanneries were included in the study by Randall and Gibson (1987). These were all located in Southern Ontario, Canada. No information was given

[5] As airborne particles have irregular shapes, the qualities that affect how easily they move through the air are expressed in terms of an idealized spherical particle. Thus, the aerodynamic diameter of an irregularly shaped particle is defined as the diameter of a spherical particle with a density of 1000 kg/m^3 and the same settling velocity as the irregular particle.

regarding the specific compounds used in the tanneries, but the authors stated that in the leather tanning industry, the tanning compounds contain Cr(III) almost exclusively rather than Cr(VI). Area air samples were collected onto PVC membrane filters from 3 different locations in each of the tanneries for 4 hours/day over 3 days. Air sampling locations were not stated explicitly and may not have been the same for each tannery. However, biological samples were collected from workers in the tanning, pressing/wringing, sorting, splitting/shaving, buffing, finishing, plant services, and supervising areas. Therefore, it is likely air sampling occurred in these worker areas. Method 7600 of the National Institute for Occupational Safety and Health (NIOSH, 1984) was used for sampling and Cr(VI) measurement. Afterward, filters were ashed and reconstituted in nitric acid for analysis of Cr_T via flame atomic absorption spectrophotometry.

Detailed results were not provided. Cr(VI) levels were reported as below the LOD. The LOD was not stated by the authors, but Method 7600 has an estimated measurement LOD of 0.05 µg/sample. TWA Cr_T concentrations did not differ among the different tannery areas, and all levels fell below 0.5 mg/m^3 (500 µg/m^3), the threshold limit proposed by the Occupational Health and Safety Division of the Ontario Ministry of Labour at the time of the analysis. TWA Cr_T exposure was reported as 1.7 ± 0.5 µg/m^3 (mean$_A$ ± SD), but the averaging time was unclear to OEHHA. Given undetectable Cr(VI) levels, the calculated concentration of Cr(III) = Cr_T.

A summary of the occupational exposure concentrations reported by Kiilunen (1983), Aitio (1984), Cavalleri (1985), Randall (1987), and their respective colleagues is provided in Table 3 below.

Table 3. Summary of personal (breathing zone) occupational exposure levels of total and trivalent chromium.

Reference	Occupational Facility Type	Subject Occupation (n)	Average (Range) Cr_T µg/m^3	Average (Range) Cr(III) µg/m^3
Kiilunen *et al*. (1983)	Cr(III) lignosulfonate production	Product packers (n = 5)	42 (2 – 230)[a]	42 (2 – 230)[ab]
Aitio *et al*. (1984)	Cr(III) leather tanning	Hide-feeders (n = 2)	13 (4 – 29)[c]	NT
		Hide-receivers (n = 4)	2 (1 – 3)[c]	NT
Cavalleri and Minoia (1985)	Welding	Welders (n = 42)	NA (21 – 225)[d]	NA (5 – 45)[d]
	Unstated Cr(III)	$Cr_2(SO_4)_3$ worker (n = 15)	NA (48 – 1700)[d]	NA (46 – 1689)[d]
	Unstated Cr(VI)	$K_2Cr_2O_7$ worker (n = 22)	NA (18 – 312)[d]	NA (10 – 100)[d]
Randall and Gibson (1987)	Cr(III) leather tanning		<500[e]	<500[be]
CAL/OSHA PEL (1976)	All under its jurisdiction	Not applicable	None	500[d]

Table summarizes occupational total and trivalent chromium exposures from peer-reviewed publications as compared to the 8-hour time-weighted average (TWA) exposure limit set by the California Occupational Safety and Health Administration (CAL/OSHA).
Abbreviations: Cr_T = total chromium; Cr(III) = trivalent chromium; Cr (IV) = hexavalent chromium; NA = not available; NT = not tested; PEL = Permissible Exposure Limit
(a) OEHHA believes these are 3-day, not 8-hour TWAs.
(b) Values assumed by OEHHA given tests by the study authors indicating all Cr in collected samples was in the trivalent oxidation state.
(c) OEHHA believes these are 6-hour TWAs.
(d) These are 8-hour TWAs.
(e) The reported value is from area samples. OEHHA believes these are 4-hour TWAs.

4. Toxicokinetics and Toxicodynamics

While some consider Cr(III) to be an essential trace element in mammals through its involvement in lipid and glucose metabolism (US EPA, 2016b), others believe there are no concrete mechanisms that define Cr(III) as essential (DesMarias and Costa, 2019; Levina and Lay, 2019). The toxicokinetics of Cr(III), i.e. the ways in which it is absorbed, distributed, metabolized, and excreted, are variable. Factors that play significant roles in the absorption, distribution, metabolism, and excretion (ADME) of Cr(III) include but are

not limited to physicochemical aerosol characteristics (e.g. size, surface area, and water-solubility), exposure routes, doses, dose rates, and nutritional status.

4.1 Absorption

Upon inhalation, Cr(III) could encounter several common fates (Schlesinger, 1988). Deposition in the head and conducting airways (trachea, bronchi, and terminal bronchioles) may involve sneezing, nose-blowing, or mucociliary clearance[6] to the pharynx for swallowing and ultimate excretion via feces. This is primarily seen with water-insoluble Cr(III) particles with an aerodynamic diameter (d_a) > 5 µm. Alternatively, with water-soluble Cr(III), d_a > 5 µm, deposition could lead to dissolution and translocation to systemic circulation through the mucus.

The Cr(III) aerosols that deposit in the gas exchange regions (respiratory bronchioles, alveoli) of the lungs can also undergo different fates. These include but are not limited to 1) uptake by macrophages, which a) exit the body via mucociliary and fecal pathways, or b) migrate to lymph nodes, lymphatic circulation, systemic (blood) circulation, and/or other extrapulmonary regions; 2) migration as in 1b without uptake by macrophages; or 3) accumulation in the lungs. Water-insoluble Cr(III) species could accumulate over time with continuous exposure and slow systemic absorption. While the Cr concentration in extrapulmonary tissues has been shown to decrease with age, the concentration in the lungs tends to increase with age (EPA, 1984; WHO, 2000). According to US EPA (1984), this increase is likely due to deposition and retention of insoluble Cr from inhaled environmental air and tobacco smoke. More soluble Cr(III) species are rapidly absorbed into the blood and translocated to other organs. Water-soluble Cr(III) species that bind proteins in the lungs could also undergo greater retention and slower absorption (Schlesinger, 1988).

4.2 Distribution

One example of Cr(III) binding to endogenous transport proteins includes its interaction with chromodulin, also known as LMWCr (low molecular weight Cr binding substance). LMWCr is an oligopeptide complex containing four chromic ions. It has been shown to transport Cr(III) from the lungs to extrapulmonary sites in the body (Wada *et al.*, 1983). According to research by Wada *et al*. (1983), after exposure to an aerosol of Cr(III) chloride hexahydrate ($CrCl_3 \times 6H_2O$), Cr burdens in the lungs of male Sprague-Dawley rats were 8-25 times that in the liver, with lung LMWCr significantly ($p \leq 0.05$) correlated

[6] Mucociliary clearance is a primary defense mechanism of the lung in which exogenous particles get trapped in the mucous lining the nasal passages and conducting airways, and swept toward the throat for swallowing by the hair-like projections (cilia) of underlying cells.

to liver levels of Cr_T, LMWCr, and HMWCr (unidentified high molecular weight Cr binding substances). Cumulative results suggested to the authors that 1) LMWCr in the lungs is in equilibrium with Cr in the rest of the body; 2) LMWCr participates in the movement of Cr from the lungs to other organs; and 3) Cr(III) accumulation in the lungs may be due to slow LMWCr synthesis in the lungs.

Several occupational (Kiilunen *et al.*, 1983; Cavalleri and Minoia, 1985; Randall and Gibson, 1987) and animal (Henderson *et al.*, 1979; Wiegand *et al.*, 1984; Edel and Sabbioni, 1985; Vanoirbeek *et al.*, 2003) studies have shown that inhaled Cr(III) compounds can be absorbed into systemic circulation. These studies are summarized in Sections 4.6 and 4.7 of the present document, respectively. Systemic absorption is influenced by the physicochemical properties of the Cr(III) compound (e.g. solubility and size; Visek *et al*., 1953), as well as its interactions with components of the biological milieu (e.g. macrophages, airway and alveolar epithelial cells, and cytosolic proteins). At least two occupational studies (Kiilunen *et al.*,1983; Aitio *et al.* 1984) indicated approximately 2-fold greater partitioning into plasma versus whole blood in general.

Once absorbed into the bloodstream, Cr(III) does not readily cross red blood cell (RBC) membranes but does bind directly to transferrin (Tf). Tf is a high-molecular-weight (80-kilodalton) primary iron (Fe)-binding blood plasma glycoprotein that controls the level of free Fe in biological fluids, and transports Fe throughout the body (ATSDR, 2011). Generally, Tf complexes with Fe(III) in blood and binds to external Tf receptors on the cell surface to initiate endosomal transport of the Fe(III)-Tf complex and cellular uptake of Fe. Fe(III) is reduced to Fe(II) and dissociated from Tf prior to entry into the cytoplasm while Tf is recycled, endosomally transported, and released to exit the cell surface (BWH, 2001).

Experiments using human hepatoma (liver cancer) cells, which have high levels of Tf receptors, indicated that Cr(III) binding to Tf blocks cellular Cr(III) uptake (Levina *et al.*, 2016). The results suggested to the study authors that the exclusion and efflux of Cr(III)-Tf complexes from cells were caused by 1) lower affinity of Cr(III)-Tf for cellular Tf receptors relative to Fe(III)-Tf complexes; 2) disruption of Cr release under endosomal conditions; and 3) disturbance of post-endosomal Tf dissociation from the receptor during recycling. Thus, Cr(III)-Tf binding may serve as a protective mechanism blocking Cr(III) accumulation in cells.

However, other studies indicated that Cr(III) binding to Tf and accumulation in tissues were related in part to the Fe status of the individual. For example, excess levels of Fe(III) were shown to impede the abilities of Cr(III) to bind Tf *in vitro* (Quarles *et al.*, 2011) and concentrate in the serum, liver, and kidneys in female rats (Staniek and Wójciak, 2018). At least one report (Feng, 2007) stated that there was a Cr transport

pathway that begins with transfer of Cr by Tf from the bloodstream into the tissues, release and processing of Cr in the tissues to form LMWCr, excretion of LMWCr back into the bloodstream, and clearance of Cr as LMWCr via the urine.

Inhaled and intratracheally instilled slightly water-soluble Cr(III) species have been shown to distribute widely in extrapulmonary tissues such as the gastrointestinal (GI) tract, bone, kidney, and liver, where accumulation is highest in the first 24 hours (Henderson *et al.*, 1979; Edel and Sabbioni, 1985; discussed in Section 4.7). Absorption via the GI tract is generally poor.

4.3 Metabolism

Toxicity of Cr(III) may be better understood through findings of Cr(VI) studies. Cr(VI) exists as the chromate oxyanion (CrO_4^{-2}) under physiological conditions (Costa and Murphy, 2019). Due to structural similarities with sulfate (SO_4^{-2}) and phosphate (PO_4^{-3}), CrO_4^{-2} is actively transported into cells non-specifically via SO_4^{-2} and PO_4^{-3} anion transporters (DesMarias and Costa, 2019). Once inside the cell, Cr(VI) undergoes rapid step-wise reductions to Cr(V), Cr(IV), and ultimately Cr(III) via enzymatic and non-enzymatic antioxidants. Ascorbate, reduced glutathione, and cysteine account for more than 95% of the Cr(VI)-to-Cr(III) conversion. Other intracellular reducing agents include, but are not limited to, cytochrome P450 reductase, mitochondrial electron transport complexes, glutathione reductase, and aldehyde oxidase (Sun *et al.*, 2015). Hydrogen peroxide (H_2O_2) and other ROS are produced during the reduction process.

Free intracellular Cr(III) cations are able to produce intracellular ROS through direct reactions with cellular molecules or indirect reactions through cellular stimulation (Wise *et al.*, 2019). Hydroxyl radicals ($^{*}OH$) and hydroxide ions (OH^{-}), for example, can be produced by Cr(III) through interactions with H_2O_2 and superoxide radicals ($^{*}O_2^{-}$) in Haber-Weiss reactions (Equations 1-2, below; Wise *et al.*, 2019; Figure 1).

Equation 1: $Cr(III) + {}^{*}O_2^{-} \rightarrow Cr(II) + {}^{*}O_2^{-}$

Equation 2: $Cr(II) + H_2O_2 \rightarrow Cr(III) + {}^{*}OH + OH^{-}$

Cr(III) and ROS can complex with ligands and attack cell membrane lipids and proteins to decrease the antioxidant capabilities of the cell and/or produce toxic responses related to oxidative stress (ATSDR, 2011; Długosz *et al.*, 2012). Such responses could include health effects like chronic inflammation and cytotoxicity (Balamurugan *et al.*, 2002; Wise *et al.*, 2019).

In some cases, Cr(III) may be further reduced to Cr(II), and undergo subsequent reactions to produce Cr(V/IV) complexes, Cr(VI), hydrogen peroxide (H_2O_2), and

organic radical species that cause oxidative DNA damage. However, this process is speculative and based on exposure to Cr(III) complexes with aromatic ligands, e.g. with supplementation of Cr picolinate (Costa and Murphy, 2019).

Still, in contrast to the ease at which Cr(VI) enters cells, ligand-bound Cr(III) is believed to enter via phagocytic or nonspecific diffusion mechanisms. Accordingly, diffusion accounts for approximately 1% of ingested Cr(III) with the other 99% being excreted in feces (DesMarias and Costa, 2019). Therefore, while intracellular accumulation of Cr(III) is the primary mechanism of Cr(VI) genotoxicity, extracellular conversion of Cr(VI) to Cr(III) is primarily viewed as a detoxification step (ATSDR, 2012; Sun *et al.*, 2015). Due to binding of Cr(III) by LMWCr, HMWCr, and Tf, Cr(III) is generally excluded from the intracellular space and precluded from inducing toxic oxidative stress responses comparable to Cr(VI), given similar *in vivo* exposures.

4.4 Excretion

Excretion of water-soluble and -insoluble Cr(III) species occurs primarily via urine and feces (Onkelinx, 1977; Henderson *et al.*, 1979; Kiilunen *et al.*, 1983; Cavalleri and Minoia, 1985; Edel and Sabbioni, 1985; Randall and Gibson, 1987; discussed in Sections 4.6 and 4.7). While most ingested chromium is excreted unabsorbed in feces, approximately 50% of absorbed chromium is excreted in the urine, about 5% is excreted in feces, and the rest is deposited in deep body compartments like bone and soft tissue (EPA, 1983; WHO, 2000; IOM, 2001). Urinary Cr(III) excretion has been reported as directly related to Cr(III) inhalation in some occupational studies (Kiilunen *et al.*, 1983; Aitio *et al.*, 1984; Randall and Gibson; 1987). However, factors such as the Cr(III) species, and experimental methodologies such as the time and frequency of urinary Cr(III) measurement relative to exposure, can produce differences within and between studies. Absorbed chromium is eliminated from the body in a rapid phase representing clearance from the blood, and a slower phase representing clearance from tissues (EPA, 1983; WHO, 2000). Two occupational exposure studies (Kiilunen *et al.*,1983; Aitio *et al*., 1984) suggested that renal excretion of approximately half of the exposure dose took <12 hours.

4.5 Physiologically-based Pharmacokinetic Models for Humans

OEHHA did not find any physiologically-based pharmacokinetic (PBPK) models that allowed for comprehensive predictions of ADME in humans inhaling Cr(III) compounds. However, one study (O'Flaherty *et al*., 2001) did allow for estimation of an upper limit based on pulmonary absorption of inhaled Cr.

O'Flaherty et al. (2001)

The human PBPK model described by O'Flaherty *et al.* (2001) was based on previously developed models of metal kinetics in humans and rats. The previous models were based on the following.

1. Movement of bone-seeking elements (i.e. lead) into and out of the skeletal tissue and bones of developing rats from birth to adulthood (O'Flaherty, 1991a; 1991b). The modelled predictions from the latter study were compared with data from a drinking water study, in which rats of different ages were chronically exposed to lead for 3-12 months until they were 440 days old.

2. Movement of lead into and out of skeletal tissue and bones of developing human adults (O'Flaherty, 1991c; 1993). Predictions from the model were compared to lead drinking water and inhalation studies in adults. Later refinements (O'Flaherty, 1995) were made to better model lead kinetics in childhood. Predictions for children were compared to several studies on lead exposure, primarily via ingestion.

3. Cr(III) and Cr(VI) kinetics in the rat (O'Flaherty, 1996; discussed in Section 4.7). The model was calibrated using data sets from oral and intratracheal exposure studies in rats given soluble Cr(III) and Cr(VI) salts. The intratracheal exposure study was that by Edel and Sabbioni (1985) discussed in Section 4.7. Predictions were compared to a study in which rats were exposed by inhalation to a Cr(VI) salt. Results of the comparisons showed that the model overpredicted Cr concentrations in blood during exposure, but fit fairly well with the post-exposure data. However, the authors acknowledged important uncertainties regarding the bioavailability/absorbability of Cr from environmental sources, and the importance of bone as a reservoir and continuing source of internal exposure to Cr.

The 2001 model by O'Flaherty *et al.* was meant for ingestion of Cr(III) and Cr(VI), and data from drinking water studies were used to calibrate the model. The model did not include a physiologic lung compartment due to lack of sufficient inhalation data, and complicating factors inherent to pulmonary Cr kinetics including compound- and particle-dependent differences. However, it did allow for estimation of impacts due to the percentage of Cr(III) absorbed by the lungs and/or the fractions of inhaled Cr remaining in the lungs and transferred to the gastrointestinal tract via swallowing.

4.6 Toxicokinetic Studies in Humans

Toxicokinetic studies in humans suggest that inhaled water-soluble Cr(III) species are absorbed into systemic circulation, where they partition into plasma versus RBCs. At

least two studies (Kiilunen *et al.*, 1983; Aitio *et al.*, 1984) reported approximately two times greater partitioning of Cr(III) into plasma versus whole blood. These studies also indicated that excretion via the kidneys is fairly rapid; estimating that it took less than12 hours for half of the inhaled Cr(III) to be excreted via the kidneys ($t_{1/2\text{-}U}$).

Kiilunen et al. (1983)

Along with the personal air samples discussed in Section 3.4.2, Kiilunen *et al*. collected urine and blood from five workers in the packing department of a Cr(III) lignosulfonate production facility.

Over three consecutive workdays, all excreted urine was collected in four portions per day. Blood samples were drawn on the first and third workdays, at the start and middle of the day, respectively. Over the following six non-workdays, morning spot urine samples were collected. All urine collection took place after workers changed clothes and showered in a building separate from the factory. Urinary Cr_T was measured by ET-AAS.

In the group of subjects, urinary Cr_T ranged from 0.01 – 0.59 µmol/L, and individual averages ranged from 0.02 – 0.23 µmol/L. Individual fluctuations of urinary Cr_T appeared to correspond to measured air exposure concentrations once the use of protective face masks was considered. However, inter-individual differences were evident in the amount of Cr excreted relative to the exposure concentration. This is to be expected, given the inhaled amount could differ based on physiological factors like breathing rate.

Peak excretion appeared toward the end or immediately after an exposure period indicating to the authors that the inhaled Cr was rapidly absorbed into systemic circulation and excreted via the kidneys. However, Cr_T in whole blood was less than the 0.02-µmol/L LOD irrespective of the collection time point. The excreted fraction in urine was calculated by Kiilunen *et al*. as 1-2% of the inhaled amount. The authors did not discuss the distribution of the other 98-99% of inhaled Cr, but it is possible much of it was swallowed and excreted through feces as suggested by studies in animals (Henderson *et al*., 1979; Edel and Sabbioni, 1985; discussed in Section 4.7). Over the seven PE days, urinary Cr_T dropped allowing the study authors to estimate $t_{1/2\text{-}U}$ was between 4 – 10 hours.

Aitio et al. (1984)

In an attempt to determine the exposure parameters that correlated best with urinary excretion and blood levels of Cr, Aitio *et al*. (1984) performed several different field and

laboratory experiments with biological samples from Finnish leather tannery press workers and themselves, respectively.

Urine was collected at variable intervals, 2-6 times/day, for seven consecutive days from the six tannery workers mentioned previously (Section 3.4.2) – two male hide-feeders and four hide-receivers of unknown sex – to examine work-related variability of total Cr. Spot urine samples were also collected from the press operators after a 10-day vacation, and before and after a 40-day vacation. Though workers used protective gloves and aprons during their work-shifts, urine collection occurred at the worker's home when possible, or in a separate building at the factory, and only after the worker had showered and changed clothes to avoid sample contamination. All urinary Cr values were normalized by creatinine excretion to account for variable hydration in test subjects.

Venous blood was collected to determine the accumulation of Cr_T in whole blood and plasma, but reporting of the collection schedule varied. Though it is clear to OEHHA staff that at least one collection occurred toward the end of the workweek (Friday morning); it is unclear, due to variable reporting by Aitio *et al*., whether the first collection day was Monday or Wednesday and whether morning and afternoon samples were taken on each of the collection days.

The field-experiment results revealed a potential for inter- and intra-personal urinary Cr_T variability associated with work tasks and work shifts, respectively. Similar to the task-driven patterns observed in personal air samples, urinalysis results showed maximal 26-fold higher urinary Cr_T concentrations in hide-feeders versus -receivers. The ranges were 0.1 – 1.3 µmol Cr/L urine versus <0.05 µmol Cr/L urine, respectively. In the two feeders, workshift-driven differences were evident in diurnal fluctuations, with generally lower urinary Cr_T in the morning, prior to workshifts, versus the afternoon. There were also urinary Cr_T concentration differences in individual feeders on different workdays, and between feeders on the same day, but Aitio *et al*. (1984) were not able to correlate these differences to breathing-zone air.

Due to the way in which the urinary data were presented by Aitio *et al*. (1984), it was difficult for OEHHA staff to accurately determine the rates at which Cr was eliminated from tannery-worker urine after the workday exposures ended. However, dramatic overnight drops in urinary Cr_T after high occupational exposures (i.e. those yielding peak urinary Cr_T concentrations ≥1.2 µmol/L) suggested the time it took for approximately half of the exposure dose to be excreted was less than12 hours.

Despite this, in feeders, a minimum baseline concentration of approximately 1 µmol Cr_T/L urine was maintained over short non-exposure periods (e.g. weekends). After 10-

and 40-day vacations, urinary Cr_T was measured at 0.2 µmol/L (10 µg/L) and ≥0.093 µmol/L (4.8 µg/L), respectively – levels reportedly 100 times higher than those seen in the non-exposed population in Finland at the time of the report suggesting some Cr accumulation/retention may have occurred. However, pre-vacation levels were not reported.

Analysis of blood plasma revealed Cr_T levels below the LOD (0.02 µmol/L; 1 µg/L) in hide-receivers; whole-blood Cr was not reported for this group of workers. In the two hide-feeders, plasma and whole-blood Cr_T levels ranged from 0.2 - 0.25 µmol/L and 0.09 – 0.13 µmol/L, respectively, in one worker and 0.34 - 0.42 µmol/L and 0.16 – 0.21 µmol/L, respectively, in the other. These results indicate approximately 2-fold greater partitioning into plasma versus whole blood in general.

The laboratory experiments involving the study authors' biological samples were aimed at measuring dermal Cr(III) absorption upon contact with tanning solution; GI Cr(III) absorption upon ingestion of Cr(III) chloride (specific compound not specified) in water; and distribution of Cr(III) and Cr(VI) upon addition to blood *in vitro*. The authors reported that dipping one hand in tanning solution for one hour (n = 1) yielded no increase in urine or blood concentrations of Cr over the 24-hour post exposure (PE) monitoring period, and no differences in blood Cr drawn from the contact versus no-contact arm.

Though not explicitly stated, OEHHA assumed the authors meant there were no changes in blood or urine Cr_T, Cr(VI), or Cr(III) concentrations after the dermal absorption test. The results suggested to the authors that no dermal absorption occurred. However, the urine and blood collection frequencies were not stated, and the low number of subjects added uncertainty to the reported results.

While OEHHA agrees dermal absorption was likely negligible in the study by Aitio *et al*. (1984), this position was informed by cumulative research (ATSDR, 2012) suggesting Cr(III) absorption via intact skin is poor and less than that of Cr(VI). Although quantitative measurements are scant, the latter was measured at approximately 3.3×10^{-5} to 4.1×10^{-4} µg/cm^2 skin per hour with a 3-hour immersion in a warm (99 ± 2.5 °F) aqueous bath of $K_2Cr_2O_7$, a Cr(VI) salt, at 22 mg/L (Corbett *et al.*, 1997). In a hypothetical situation in which a worker had both hands (1070 cm^2 skin; EPA, 2011) immersed in a similar solution for 1 hour, the maximum amount of Cr(VI) absorbed would be 0.44 µg (0.00041 µg/cm^2-hour × 1070 cm^2 × 1 hour), assuming intact skin. Dermal absorption of a Cr(III) solution is expected to be even less than that.

In the GI absorption experiment (n = 2), wherein urine was collected every 6 hours for 24 hours, ingestion of 5 mg (96 µmol) Cr(III) in 100 mL water (960 µmol/L) by the researchers yielded peak urinary Cr_T (>0.02 µmol/L) at 6 hours PE and negligible levels

at 24 hours PE, with Cr_T recovery approximately 0.17% (0.16 µmol) of the administered dose. According to the Agency for Toxic Substances and Disease Registry (ATSDR, 2012), it is typical for ≤1% of an orally administered Cr(III) dose to be recovered in urine of animals and humans, with >95% of the dose excreted via feces. No explanation was provided by Aitio *et al*. for the distribution of the rest of the administered dose, and the low number of subjects added to the uncertainty of the reported results. However, fecal elimination likely accounted for the vast majority of the ingested dose (ATSDR, 2012).

Given urinary data from GI absorption and occupational experiments, the inability to correlate inter- and intra-personal urinary Cr_T differences to inhalation exposures, and the TWA Cr_T exposure concentrations (<20 µg/m^3) measured for the hide-feeders, the authors believed that incidental ingestion of tanning liquid (e.g. via splashes on the face) could reasonably explain some variability in the renal excretion patterns of hide-feeders.

In vitro testing of blood drawn from a non-exposed individual, spiked with Cr(III) chloride or chromic (VI) oxide to a final concentration of 0.35 umol/L (18 µg/L), diluted with 0.9% sodium chloride (NaCl) to a hematocrit[7] level of 0.30, and allowed to stand at "room temperature" for 1 hour yielded plasma-to-cell ratios of 32:1 and 0.67:1 for Cr(III) and Cr(VI), respectively. These results supported the idea that the partitioning of Cr(III) is much greater in plasma, while that of Cr(VI) is greater in cells. This idea is further supported by additional *in vivo* and *in vitro* reports (Wiegand *et al.*, 1984; Cavalleri and Minoia, 1985; Edel and Sabbioni, 1985; P. Coogan *et al.*, 1991; Ducros, 1992; Vanoirbeek *et al.*, 2003) of limited Cr(III) uptake by RBCs relative to Cr(VI), within the first 24-48 hours PE.

Cavalleri and Minoia (1985)

As mentioned in Section 3.4.2, Cavalleri and Minoia (1985) examined urine and/or blood of 79 workers. Group A (n = 42) was exposed to Cr during welding operations, Group B (n = 15) was exposed to $Cr_2(SO_4)_3$ and some Cr(VI), and Group C (n = 22) was exposed to $K_2Cr_2O_7$ PM, chromic acid fumes, and chromic acid PM. Urine was collected before and after one 8-hour work shift, and analyzed immediately after each collection to avoid post-collection reductions of Cr(VI) to Cr(III). Blood was collected from 16 workers — 7 from Group B and 9 from Group C (chromic acid-exposed) — for quantification of Cr in whole blood, serum, and RBCs.

Recognizing the potential experimental error that could be introduced by the interconversion of Cr(III) and Cr(VI) in collected samples, Cavalleri and Minoia (1985) employed the use of ET-AAS with Amberlite LA-1 or -2 anion-exchange resins activated

[7] Hematocrit is the ratio of the volume of red blood cells to the total volume of blood.

in an unspecified organic solvent. These resins are positively charged, so they attract and remove anions (negatively charged ions) from solution. Given that Cr(III) and Cr(VI) exist in solution primarily as cations and anions, respectively, the resin would enable the isolation of the two species after collection and prior to analysis by ET-AAS.

According to the authors, the method enabled more accurate measurements of Cr species in biological samples by eliminating the need for complex sample preparations that could result in contamination and/or changes in Cr valence states and allowing the rapid separation of Cr(VI) from various biological matrices. The reported limit of detection for the method was 0.1 µg/L in previous experiments with Cr-spiked rat urine.

Urinary Cr_T ranged from 37 ± 12 µg/L in Group A, 24.7 ± 19.3 µg/L in Group B, and 31.5 ± 16.3 µg/L in Group C. The absence of urinary Cr(VI) in all groups suggested that the measured Cr_T in urine was Cr(III), but the authors couldn't pinpoint the biological compartment in which the reduction occurred.

The urinary Cr(III) levels did not reflect occupational exposures to Cr(III). Group B subjects who were exposed to the highest concentrations of Cr_T and Cr(III) appeared to have the lowest urinary levels. These results align with others (Edel and Sabbioni, 1985) that indicate slower translocation of Cr(III) compounds from the lungs versus Cr(VI) compounds. Calculations[8] by OEHHA, assuming a breathing rate of 10 m^3/day (OEHHA, 2008), alveolar deposition of all the inhaled Cr, urinary excretion of 2 L/day (MedlinePlus), and a workday of 8 hours suggest the excreted fraction of Cr in urine in Group B was less than 1% - 6% of the inhaled amount, which overlaps with the estimate by Kiilunen *et al*. (1983).

Randall and Gibson (1987)

Randall and Gibson collected urine and blood from 124 male tannery workers and control subjects to determine whether serum and urinary Cr levels could be used as indices of Cr exposure in the former group. The tannery workers (n = 72) were 36 ± 12 years of age (mean ± SD) and came from four different facilities in Southern Ontario. Length of employment in the tanning industry ranged 1-48 years with a mean of 10.6 years. The control workers (n = 52) were 41 ± 13 years of age (mean ± SD), from the Guelph and Toronto areas of Ontario, and not occupationally exposed to Cr. Details

[8] Exposure levels in Group B were measured at 48-1700 µg/m^3. The Cr 8-hour workday inhalation dose (Cr_I) = breathing rate (10 m^3/day) × exposure concentration = 480 – 17,000 µg/day. Using the average urinary excretion of Cr_T in Group B (24.7 µg/L), the amount of Cr excreted after an 8-hour workday (Cr_U) = 24.7 µg/L × daily volume of urine produced (2 L/24 hours) × hours worked/day (8 hours/day) = 16.5 µg/day. Thus, the fraction of inhaled Cr_T excreted in urine after an 8-hour workday = Cr_U / Cr_I x 100, or 0.1% - 6.1%.

were not provided regarding the work environments or occupations of the controls. Individuals in the tannery and control groups were matched by age, race, and socioeconomic status. According to the study authors, each subject was healthy with no history of insulin- or noninsulin-dependent diabetes or coronary heart disease, and no dietary supplementation of Cr or yeast.

Whole blood samples were collected from overnight-fasted individuals (n = 124) on Tuesday mornings and allowed to clot for collection of serum. Spot urine samples were collected from 49 tannery and 43 control workers on a Friday afternoon, and from 42 tannery workers on the following Monday morning. Urinary creatinine content was determined to account for variable hydration in test subjects. Non-parametric (Kruskal-Wallis) tests were used to determine differences between tannery and control workers, and between tannery workers from different areas of the tanneries. However, due to the limited number of examined time-points, OEHHA was unable to determine the rates of Cr(III) elimination from urine.

Comparisons between tannery and control workers showed median serum Cr, urinary Cr, and urinary Cr-to-creatinine ratios were over three times higher in the former versus the latter group (p = 0.0001 for all endpoints). In control subjects, but not tannery workers, serum Cr levels were correlated with age (r = 0.29; p = 0.03). There were no significant correlations between urinary Cr or the Cr-to-creatinine ratio and age, height, or weight of either the tannery or control workers.

In tannery workers, Tuesday morning serum Cr values were better correlated with urinary Cr-to-creatinine ratios from Friday afternoon samples (r = 0.72; p = 0.001) than the following Monday morning samples (r = 0.45; p = 0.003). While comparisons of tannery workers from various departments showed that TWA Cr_T exposures did not differ ($mean_A \pm SD = 1.7 \pm 0.5$ μg/m^3), there were statistically significant ($p < 0.05$) differences in serum and urinary Cr. Workers in the tanning and pressing/wringing areas (Group 1) had higher serum Cr_T and urinary Cr-to-creatinine ratios than workers in the sorting, splitting/shaving, and buffing areas (Group 2), and the finishing, plant services, and supervisor areas (Group 3). Median Tuesday morning serum Cr_T levels were more than two-times higher in Group 1 (1.04 ng/mL) than Groups 2 and 3 (0.44 ng/mL and 0.39 ng/mL, respectively). Median Friday afternoon urinary Cr-to-creatinine ratios were approximately five-times higher in Group 1 (2.75 ng/mg) than Groups 2 and 3 (0.61 ng/mg and 0.54 ng/mg, respectively).

By the following Monday morning, the median urinary Cr-to-creatinine ratio was nearly four-times lower than on Friday (0.78 ng/mg versus 2.75 ng/mg) in Group 1, but fairly unchanged in the other two groups. Despite this, the Group 1 Monday morning ratio was still significantly ($p < 0.05$) higher than those of Groups 2 and 3. Though it is likely

the Cr loss exhibited in Group 1 was due to elimination, the lack of weekend urine samples precluded confirmation. There were no correlations between the biological endpoints of tannery workers and length of employment. Personal hygiene, accidental ingestion, use of personal protective equipment, and promotions to management positions were acknowledged as factors affecting occupational Cr absorption in the tannery workers.

4.7 Toxicokinetic Studies in Animals

OEHHA did not find any publications on animal PBPK models that were used for extrapolation of human ADME parameters for inhaled Cr(III). However, experimental studies in animals suggest that once in the lungs, water-soluble Cr(III) compounds can demonstrate poor diffusability across alveolar membranes (Edel and Sabbioni, 1985). This, along with binding to high-molecular-weight components in the lung cytosol (Edel and Sabbioni, 1985), and slow cellular uptake via non-phagocytic mechanisms, contributes to slower translocation from the lungs to extrapulmonary tissues relative to Cr(VI). Once absorbed into systemic circulation, Cr(III) was shown in animals, like in humans, to partition to a greater extent into plasma versus whole blood or RBCs (Wiegand *et al.*, 1984; Edel and Sabbioni, 1985; Vanoirbeek *et al.*, 2003).

(Onkelinx, 1977)

Onkelinx performed a compartmental analysis of Cr(III) metabolism in female Wistar rats intravenously exposed to “trace” amounts of isotopically-labeled Cr(III) in a single 0.25-mL injection. Rats (n = 6-8/group) were fairly young, at 35, 60, or 120 days of age at the beginning of the experiments, considering 120 days is approximately 1/6th of a rat lifetime (OEHHA 2008b). There was no mention of a control rat group. The injectant, a solution containing 150 µCi of $^{51}Cr^{+}$ and 0.76 µg of Cr, was made from $^{51}CrCl_3 \times 6H_2O$ in 0.5 M hydrochloric acid and diluted in 0.9% NaCl. The specific activity was 198,000 µCi/mg Cr, and radionuclidic purity was 99%. Radioactive determinations of $^{51}Cr^{+}$ counts were made with a reported counting error of <5%. This was the only study found by OEHHA to compare kinetics of Cr(III) in animals of different ages; no studies were found to compare sex-related differences in Cr(III) kinetics.

In kinetic experiments, radioactivity was quantified in biological samples of blood, feces, and urine. Blood samples were obtained from the tip of the tail at intervals ranging 1 hour to 11 days PE for analysis of $^{51}Cr^{+}$ in plasma. Feces and urine samples were collected over the first 3 PE days.

Analysis of blood plasma showed that $^{51}Cr^{+}$ clearance was rapid during the first 6-8 hours but slowed sequentially from 8-120 hours and time-points thereafter. Results

suggested to the study authors that elimination occurred by first-order kinetics[9] and could be modeled by a 3-compartment model. Though urinary $^{51}Cr^{+}$ elimination was highest in the 60-day old group, and fecal elimination was highest in the 35-day old group ($p < 0.05$ for each relative to other age groups). In general, results showed that irrespective of rat age, approximately half of the injected $^{51}Cr^{+}$ dose was eliminated during the first 3 PE days. Over that time period, renal (urinary) and fecal pathways accounted for roughly 90% and 10% of the total excreted $^{51}Cr^{+}$, respectively, suggesting to OEHHA that the primary (urinary) route of elimination did not change with age.

This pattern is opposite of that observed by Henderson *et al*. (1979) and Edel *et al*. (1985), suggesting to OEHHA that intravenous exposures may not be as useful as intratracheal instillation for modeling the distribution and elimination of inhaled Cr(III). This conclusion was supported by O'Flaherty (1996), who reported that tissue distribution and excretion patterns were different in intravenous versus oral and intratracheal exposures.

In serial sacrifice experiments, Onkelinx used 60-day old rats (n = 30) with an average body weight (BW) ± standard deviation (SD) of 192 ± 5.2 g were sacrificed in groups of 3-4, at intervals ranging 1 hour to 11 days PE, for quantification of $^{51}Cr^{+}$ in blood, minced organ, and lyophilized (freeze-dried) femoral tissues. Liver, spleen, pancreas, kidney, and lung tissues were examined, as were the separated epiphysis (head) and diaphysis (shaft) of the femur. While soft tissues were removed from the femurs, epiphyseal samples were composites of bone, cartilage, and bone marrow, and diaphyseal samples were cleaned of marrow such that they were pure compact bone.

As with other studies (Kiilunen *et al.*, 1983; Aitio *et al.*, 1984; Wiegand *et al.*, 1984; Edel and Sabbioni, 1985; Vanoirbeek *et al.*, 2003), Cr distributed primarily to the plasma fraction of blood and minimally to RBCs. Analysis of temporal distribution patterns in other tissues showed that from 1 hour to 11 days PE, Cr increased in epiphyseal, diaphyseal, and splenic tissues but tended to decrease in the lungs and pancreas and remain the same in the liver. Levels in the kidney were variable, with the highest levels at 1 hour and 4-11 days PE. These results suggested to OEHHA that long bones and the spleen may serve as long-term sinks for Cr, while the liver and kidney mediate the elimination of Cr via feces and urine, respectively. However, additional experiments are still needed to confirm whether these tissues would also serve as Cr(III) reservoirs upon inhalation and over similar PE timeframes.

[9] First-order elimination kinetics occur when a constant proportion (e.g. percentage) of the administered substance (e.g. $^{51}Cr^{+}$) is eliminated per unit time, and the elimination rate is proportional to the amount of said substance in the body.

Henderson et al. (1979)

Some of the earliest data on Cr(III) toxicokinetics were reported in 1979 by Henderson *et al.* In their study, two radioactive tracing experiments were performed with a gamma-emitting isotope of chromium chloride hexahydrate ($^{51}CrCl_3$ x $6H_2O$), a water-soluble salt (NCBI, 2019c), for quantification of radioactivity, and thus Cr, in biological compartments. The chemical purity and vendor were not stated. The experiments included exposure via nose-only aerosol or intragastric instillation.

For nose-only exposures, Syrian hamsters[10] of an unstated age were exposed to a nebulized $^{51}CrCl_3$ aerosol at concentrations of 0 (control; unstated carrier solvent alone), 2.8 (low), or 77 mg/m^3 (high) for 30 minutes and sacrificed at 2 hours or 1, 7, or 21 days PE. There were 4 hamsters/sex/treatment group/time-point. The aerosol had a mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) of 1.7 ± 1.7 µm.

Upon necropsy, pelt, skull, pancreas, spleen, liver, kidney, GI tract, lung, lung fluid, and carcass samples were collected for quantification of radioactivity. Doses were not estimated, and total body burden was not stated. However, initial lung burdens determined from animals sacrificed at the 2-hour time-point were 0.71 ± 0.19 µg and 20.4 ± 9.7 µg for the low- and high-exposure hamsters, respectively. According to the authors, the lung burden estimates did not include the $^{51}Cr^{3+}$ activity observed in the liver and kidney at 2 hours PE because it could be accounted for by absorption observed from the GI tract. At the 2-hour time-point, lung burden corresponded to 11.6 ± 2.1% of the total $^{51}Cr^{3+}$ in the body. Fractional burdens for other organs are shown below in Table 4.

[10] Syrian hamsters (*Mesocricetus auratus*) have been used in other studies to model the structural changes (i.e. airway remodeling) that occur in humans with chronic lung diseases like asthma, chronic obstructive pulmonary disease (COPD), and fibrosis (Wright *et al*., 2008; Talaei *et al*., 2011). Though Syrian hamsters are available in inbred and outbred strains, it is unclear to OEHHA which type was used in the study by Henderson *et al*. (1979).

Table 4. Calculated $^{51}Cr^{3+}$ Deposition in Tissues Collected from Syrian Hamsters at Two Hours Post Inhalation of a Nebulized $^{51}CrCl_3$ Aerosol.

Tissue	Fraction of Total Body Deposition (% ± %)[a]	Calculated Depositional Mass (µg ± µg)	
		Low-dose Group (2.8 mg/m^3)[b]	High-dose Group (77 mg/m^3)[c]
Pelt	30.4 ± 5.0	1.9 ± 1.5	53 ± 59
Lung	11.6 ± 2.1	0.71 ± 0.19	20.4 ± 9.7
Kidney	1.4 ± 1.4	0.086 ± 0.18	2.5 ± 6.4
Liver	1.4 ± 1.4	0.086 ± 0.18	2.5 ± 6.4
GI tract	36.1 ± 8.2	2.2 ± 2.0	63 ± 77
Depelted skull	15.4 ± 3.8	0.94 ± 0.88	27 ± 34
Carcass remains	3.7 ± 1.1	0.23 ± 0.23	6.5 ± 8.7

Table summarizes fractional deposition data from Henderson *et al.* (1979), and depositional masses primarily calculated by OEHHA. In the study, hamsters were exposed to $^{51}CrCl_3$ at 0, 2.8, or 77 mg/m^3 for 30 minutes (n = 4/sex/treatment group/time-point).
Abbreviation: GI – gastrointestinal.
(a) Values in this column were taken directly from Henderson *et al*. (1979) .
(b) Values in this column, except those for the lung, were calculated by OEHHA. For the low-dose group, reported mean ± standard deviation (SD) values for the lung burden (0.71 ± 0.19 µg) and fractional lung deposition (11.6 % ± 2.1%), at 2 hours post exposure, were used to calculate the total body burden for the low dose group. Total body burden was then used to calculate the deposited mass in various tissues of the low-dose group animals. These calculations, shown in Attachment A, assume a worst-case scenario with the largest SD.
(c) Values in this column, except those for the lung, were calculated by OEHHA in the manner similar to that described in note “b” above.

Results at the 2-hour time-point (Table 4) indicated a high degree of variability, which is visible in the reported SDs. High levels of ^{51}Cr in the pelt suggested that despite the nose-only exposure, much of the Cr ended up on the fur. Fur-grooming and swallowing of inhaled chromium could partially explain high ^{51}Cr levels in the GI tract. Nasal deposition/retention may account for the levels in the skull. It is unclear to OEHHA whether results from the low- and high-exposure groups were the same or combined to obtain the fractional organ burdens. In the former case, it would suggest to OEHHA that the pharmacokinetics were the same in the low- and high-exposure groups. At the 3-

week time-point (Figure 1), lung burden was reduced by 60% indicating some retention of Cr(III). Temporal patterns of $^{51}Cr^{3+}$ retention and distribution relative to the lung are shown in Figure 1. Associated signs of lung damage are discussed in Section 5.3 herein.

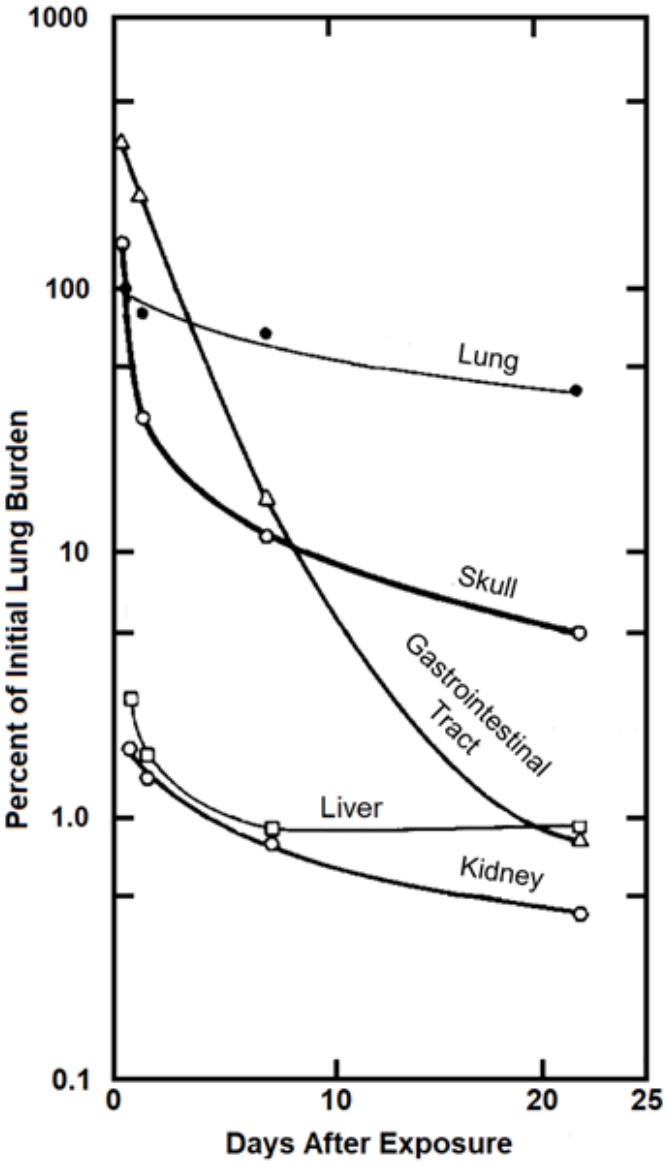

Figure 1. Retention and distribution of inhaled $^{51}CrCl_3$ in the Syrian hamster over time. The initial lung burden (ILB) was calculated from the $^{51}CrCl_3$ radioactivity in the lungs of animals sacrificed 2 hours post inhalation of 0, 2.8, or 77 mg/m3 for 30 minutes. The figure was reproduced from Henderson et al. (1979; Figure 3). The figure legend stated that ILB values of animals sacrificed at later time periods were estimated from whole-body radioactivity counts made immediately after [2 hours post] exposure.

Intragastric instillation experiments were performed with a 0.5-mL solution of water and $^{51}CrCl^3$ (0.2 ng; 0.04 µCi) administered to each of four hamsters sacrificed 4 or 24 hours post instillation (n = 2/time-point; sex not stated). At sacrifice, for each animal, the GI tract and carcass radioactivity was quantified, and the quantity of Cr ion absorbed from the GI tract was calculated. GI absorption was found to be 15.3% and 13.7% (approximately 0.03 ng) in the two hamsters sacrificed at the earlier time-point. By 24 hours PE, 97% of the originally instilled material was excreted, and less than 2% (0.004 ng) was found outside the GI tract. These results indicated distribution patterns and elimination rates differed between inhalation and intragastric exposure routes.

Cavalleri & Minoia (1985)

In vitro experiments performed by Cavalleri and Minoia (1985) with rat whole blood, plasma, and RBCs showed that reduction of an unstated dose of Cr(VI) to Cr(III) was most rapid upon addition to isolated RBCs or whole blood (Figure 2). Approximately 61%, 77%, and 86% of the added Cr(VI) remained in RBCs, whole blood, and plasma, respectively, after 20 seconds. After three minutes, <20% remained in RBCs and whole blood. No measurements were reported for plasma after the first 20 seconds.

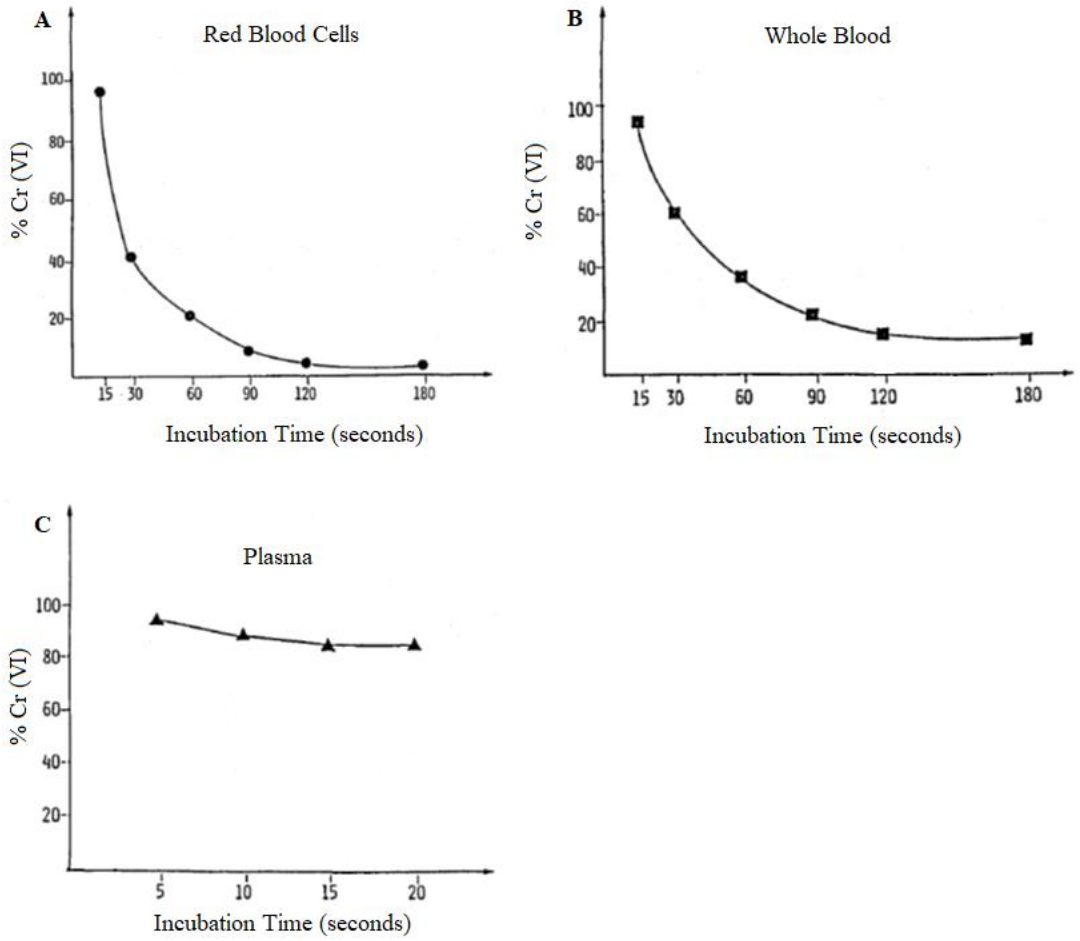

Figure 2. Reduction of Cr(VI) over time upon incubation at 37 ± 0.1 °C with rat red blood cells (A), whole blood (B), and plasma (C). The panels were compiled from Figures 1-3 of Cavalleri and Minoia (1985). OEHHA used GetData software to determine the percentage of Cr(VI) remaining over time. GetData allows users to obtain original (x,y) data from scanned scientific plots when the values are not available.

Edel and Sabbioni, 1985

In an investigation of the metabolism and excretion of Cr(III) and Cr(VI) compounds, Edel and Sabbioni (1985) intratracheally instilled outbred male Sprague-Dawley rats with 0.1 or 10 µg of $^{51}CrCl_3$ or sodium chromate ($Na_2{}^{51}CrO_4$, a hexavalent compound. The volume of the instillate was 0.1 mL or 0.001 mL, but it is unclear to OEHHA which volumes were used for the different experiments. There were 2-4 rats/group, and BW = 200-220 g suggesting to OEHHA they were young adults, possibly between 5 and 8 weeks of age. Rats exposed to 0.1 µg were sacrificed 24 hours PE for quantification of ^{51}Cr activity in various biological samples. Rats exposed to 10 µg were kept in metabolic cages with access *ad libitum* to mineral water and commercial chow for

collection of urine and feces over 7 PE days prior to sacrifice. The same types of biological samples were collected from all groups irrespective of the sacrifice time.

Results of $^{51}CrCl_3$ exposures at 24 hours PE are shown in Table 5. Those from $Na_2{}^{51}CrO_4$ exposures are not shown.

Table 5. Chromium content in rat tissues and lung lavage 24 hours after intratracheal injection of 0.1 µg of ^{51}Cr(III) per rat.

Tissue	Mean ^{51}Cr(III) Deposition ± SD (% of dose per g of tissue)
Lung	19.700 ± 1.990
Trachea	3.110 ± 1.890
Kidney	0.044 ± 0.007
Liver	0.006 ± 0.001
Spleen	0.007 ± 0.002
Epididymis	0.005 ± 0.002
Testes	0.003 ± 0.002
Femur	0.034 ± 0.003
Stomach	0.007 ± 0.003
Small Intestine	0.006 ± 0.003
Large Intestine	0.011 ± 0.003
Blood	0.010 ± 0.004
Plasma[a]	85.26 ± 2.39
RBCs[a]	14.77 ± 2.39
BALF	0.39 ± 0.097

Table summarizes data regarding site-specific deposition of radiolabeled Cr(III) and was modified from Table 1 of Edel and Sabbioni (1985), who exposed rats (n = 4) to 0.1 of radiolabeled chromium (III) chloride ($^{51}CrCl_3$). It is unknown to OEHHA whether the reported means are arithmetic or geometric. Cr(III) levels in pancreas, brain, heart, thymus, skin, fat, and muscle tissues were not determined, and the analyzed mass of each tissue type was not stated. Abbreviations: BALF = bronchoalveolar lavage; RBCs = red blood cells; SD = standard deviation.

(a) Reported values are % of total blood.

Overall, analyses by Edel and Sabbioni (1985) showed that at 24 hours PE, most of the remaining ^{51}Cr was in the lung, trachea, and BALF followed by the kidneys, which mediate urinary elimination of Cr, and the femur, which has been shown (Onkelinx, 1977) to accumulate Cr. With respect to blood components, nearly 6-fold greater partitioning of ^{51}Cr was observed in plasma relative to RBCs. This hematological pattern aligns with reports indicating poor cellular uptake of inorganic Cr(III) compounds (Wiegand *et al.*, 1984; ATSDR, 2011). Subcellular distribution of ^{51}Cr(III) in lung

homogenate was heavily skewed with the highest amounts observed in the nuclear fraction, followed by the mitochondrial, lysosomal, and cytosolic fractions. These fractions accounted for 41%, 24%, 21%, and 10% of the measured ^{51}Cr(III) in lung homogenate, respectively.

Elution of the cytosolic fraction from ^{51}Cr(III)- and ^{51}Cr(VI)-exposed rats on Sephadex G-75 gel columns revealed qualitatively similar profiles with three peaks — two corresponding to an HMWCr component and one corresponding to an LMWCr component. However, in ^{51}Cr(III)-exposed rats, most of the remaining ^{51}Cr was associated with HMWCr which cleared more slowly from the lungs. In ^{51}Cr(VI)-exposed rats, most of the remaining ^{51}Cr was associated with LMWCr, which cleared more rapidly.

Cumulative urinary and fecal excretion following instillation of 10 µg ^{51}Cr(III) was highest after the first two PE days at approximately 2% and 34% of the administered dose, respectively. By seven days PE, cumulative excretion by these routes was still only about 3.6% and >36% of the administered dose. Greater elimination via feces versus urine is supported by findings of Henderson *et al*. (1979). The authors stated that results indicated mucociliary clearance, swallowing, and digestion of inhaled Cr(III) played a greater role than absorption from the lungs. They cited unpublished work suggesting that after 7 days PE to ^{51}Cr(III), lung ^{51}Cr was much lower, but there were no significant changes in the other tested tissues. Overall, these results suggested to OEHHA that after 7 days, roughly half of the instilled Cr was still in the body, presumably in the liver, kidney, and bone.

O'Flaherty (1996)

In the 1996 PBPK model by O'Flaherty (Figure 3), general physiology, body growth, and tissue and organ growth parameters were defined using O'Flaherty's previous studies (1991a; 1991b) involving kinetics of lead and other "bone-seeking" elements (e.g. radium, strontium, and aluminum). The model was adapted to chromium by first considering the disposition of Cr(III) after intravenous administration. Subsequently, adding other routes of exposure in increasing order of kinetic complexity, and repeating the same process for Cr(VI). The features of chromium kinetics forming the basis of the 1996 model were taken from Cr(III) and Cr(VI) studies of intravenous, stomach tube, drinking water, and intratracheal instillation exposure routes. Exposure, Cr(VI) reduction to Cr(III), and distribution parameters were initially estimated using data from the aforementioned exposure studies.

Most of these studies, except that of Edel and Sabbioni (1985), are beyond the scope of the present document due to a focus on Cr(VI) or extrapulmonary routes of exposure

and are not summarized in the present document. Given the initial estimates were obtained from an intravenous exposure study, the resulting model was not ideal for predicting kinetics from more realistic routes of exposure like inhalation and oral intake. Thus, the initial estimated parameters in O'Flaherty's 1996 model were adjusted to visually match simulations of chromium in various tissues over time to data from single-dose intratracheal instillation studies. For example, a "retained urine" compartment (Figure 3) was added to account for a lag time in urinary chromium excretion over the days following exposure. However, ultimately, after calibration, the best modelled predictions of blood chromium concentrations were compared to results from a study in which rats inhaled Cr(VI), not Cr(III), 6 hours/day for 4 days.

Studies of inhaled Cr(III) were not used to calibrate or test the model, and the model was not independently verified. Absorption, excretion, and Cr(VI) reduction were modeled primarily using first-order rate constants. First-order kinetics suggests to OEHHA that the rates of these three processes are insaturable and diffusion-driven, not flow-driven, and the fraction of chromium processed per unit time is constant.

First-order rates do not account for chromium binding to transport proteins, which can be limited by factors such as the presence of other metals (e.g. iron) in the body and protein synthesis rate. Physicochemical characteristics (e.g. water solubility) and physiological/nutritional factors (e.g. fasted versus fed, dietary amino acids, and zinc status) that could affect absorption were also not taken into account in the model. Fractional absorption of chromium was recognized by O'Flaherty as a key uncertainty.

O'Flaherty also acknowledged the model did not account for the non-linear, dose-dependent kinetics observed in the liver and kidney in chronic Cr(VI) drinking water experiments. The unresolved need to understand bone as a reservoir and continuing source of internal chromium exposure was additionally mentioned as a necessary component of future (complete) models of chromium kinetics.

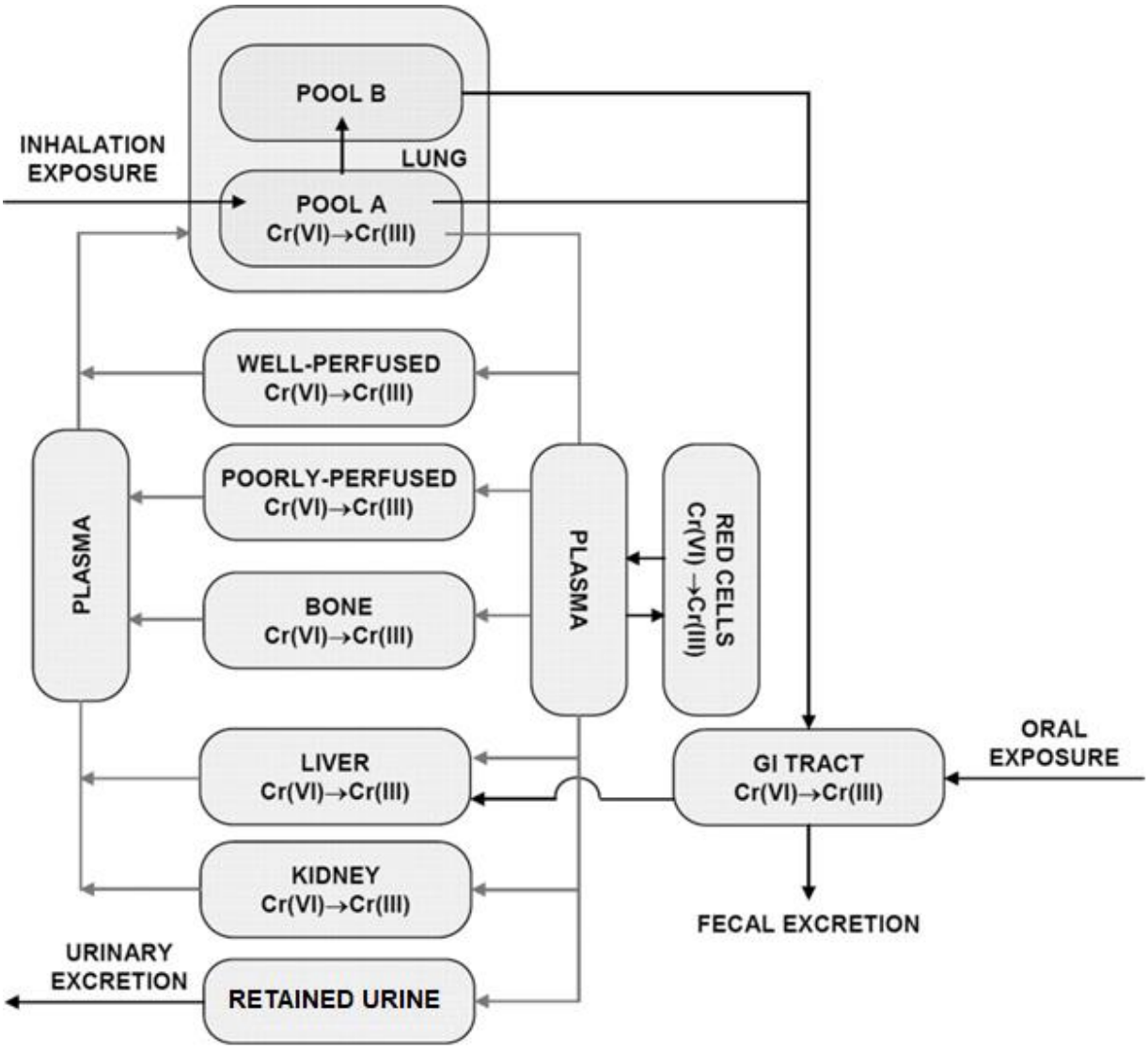

Figure 3. Schematic diagram of the chromium model by O'Flaherty (1996).
Chromium can be absorbed as a result of oral or inhalation exposure. Chromium entering the lung is deposited into bioavailable pool A, from which it can be absorbed into systemic circulation or transferred either to the gastrointestinal tract or to non-bioavailable lung pool B. Chromium in pool B can move only to the gastrointestinal tract. Chromium (VI) is reduced to Cr(III) in all tissues and gastrointestinal tract contents, but not in blood plasma. A holding compartment for urine is introduced to account for the excretion delay seen experimentally. The diagram and legend were reproduced from Figure 1 of the publication.

4.8 Species Differences in Metabolism and Elimination

OEHHA was unable to find peer-reviewed publications of original research into the comparative metabolism and elimination of Cr(III) among humans and animals. However, research described in sections 4.6 and 4.7 above suggest these processes may be similar across species. This conclusion is supported by a report from the US Agency for Toxic Substances and Disease Registry (ATSDR, 2012) which reached a similar conclusion.

5. Acute and Subacute Toxicity

5.1 Studies in Humans – Allergic Sensitization and Asthma Risk

Most of the studies into the acute/subacute toxicity of Cr(III) in humans were performed several decades ago. Earlier studies (e.g. Fregert and Rorsman, 1964; Samitz and Shrager, 1966)[11] sought to determine the cross-reactivity of Cr(III) and Cr(VI) compounds and quantify the dermal sensitization potential of Cr(III) compounds relative to others. Later studies (e.g. Novey *et al*., 1983; Park *et al*., 1994) tended to report the results of Cr sensitization tests in occupationally exposed subjects complaining of asthma and other allergy-related sequelae.

Chemical sensitization is generally recognized as a physiological change that occurs in an exposed organism and causes it to produce a stronger allergic immune reaction upon subsequent (challenge) exposures and at lower doses than would be observed in non-sensitized individuals. Chemical hypersensitivity can result in effects such as asthma, conjunctivitis, or rhinitis, or dermal effects such as urticaria. Conjunctivitis is an inflammation of the transparent membrane lining the eyelid and the white part of the eyeball. Rhinitis is inflammation and swelling of the mucous membrane of the nose characterized by runny nose, sneezing, and stuffiness. Urticaria is a skin rash characterized by itchy, raised, red- or skin-colored welts also known as hives.

Fregert and Rorsman (1964)

The study by Fregert and Rorsman primarily involved 22 test subjects who developed eczematous inflammation after topical exposure hexavalent $K_2Cr_2O_7$ (0.1 M), and had reactions to intracutaneous injections of $K_2Cr_2O_7$ (0.001 M). To test the subjects, cross-reactivity to trivalent $CrCl_3 \times 6H_2O$, skin patch and intradermal injection challenge tests were performed. In skin patch tests, the suspected allergen is applied to the surface of the skin and secured for a period of time (generally 48 hours) to test for delayed

[11] Our literature search also identified a 1966 report of an experiment conducted on prisoners at the Holmesburg Prison in Philadelphia, which is excluded due to concerns about ethics and reporting.

reactions such as allergic contact dermatitis. Intradermal injection tests were often used to test the sensitization potentials of chemicals with differing dermal penetration capabilities. In the publication by Fregert and Rorsman, few details were provided. However, no Cr(VI) contaminants were observed in the $CrCl_3 \times 6H_2O$ test materials when examined using a sym-diphenylcarbazide method capable of detecting chromate in a 1:100,000 dilution. Volunteers with no reactions to $K_2Cr_2O_7$ skin patch tests or intradermal injections were included as controls.

Patch testing was done with 0.07-M or 0.5-M $CrCl_3 \times 6H_2O$ in 22 and 17 of the test subjects, respectively. Twenty-three volunteers were included as controls and exposed to the 0.5-M solution. Positive (eczematous) reactions were observed in 4/22 test subjects (18%) exposed at the lower concentration, and 11/17 subjects (65%) tested at the higher concentration. Negative reactions were observed in the controls.

Intracutaneous injections were performed in all test subjects with 0.1 mL of 0.001-M or 0.01-M $CrCl_3 \times 6H_2O$ solutions. Ten volunteers were included as controls and exposed to the 0.01-M solution. The lower concentration produced positive reactions (i.e. skin inflammation 5-12 mm in diameter) in 12 of the test subjects (55%) while the higher concentration produced positive responses in all 22 subjects (100%). None of the controls had positive reactions.

Exudate was collected from lesions formed after intradermal injection of 0.01-M $CrCl_3 \times 6H_2O$ (n =22), and patch tests with 0.07-M and 0.5-M $CrCl_3 \times 6H_2O$ (n = 4 and 10, respectively) for quantification of basophils. Basophils are white blood cells that migrate to sites of inflammation, and release enzymes shown to play roles in infection and some types of allergic skin inflammation. Because none of the control subjects had lesions association with the Cr(III) exposures, a cantharidin solution was applied topically to cause blister formation. Basophils comprised 0-0.6% of the cell population in exudate from controls, and >1% of the cell population in 14/22, 4/4, and 9/10 exudate samples from the aforementioned experiments, respectively. The authors cited other studies to show the basophil fractions were on the same order as those in reactions to Cr(VI) compounds. According to Fregert and Rorsman (1964), their cumulative results provided unequivocal evidence that Cr(VI) allergy implies allergy to Cr(III) as well.

Samitz and Shrager (1966)

This short publication reported the results of patch test results in five chromate [Cr(VI)]-sensitive subjects challenged with $K_2Cr_2O_7$ (0.1% - 0.25%) and various Cr(III) compounds including 0.1% - 5% $CrCl_3$, 0.5% - 5% $Cr(NO_3)_3$, and 0.5 - 1% $Cr_2(SO_4)_3$. Use of equimolar concentrations of Cr(VI) and Cr(III) compounds allowed the authors to

compare cross-reactivity of the two compounds in experiments performed with intact skin.

Separate experiments with cellophane tape-stripped skin were performed in four subjects challenged with a subset of the listed Cr(III) compounds. Skin stripping is a widely used method to study the kinetics and penetration depth of drugs. It is generally achieved by removing the uppermost skin layer (stratum corneum) through repeated application of adhesive tapes. Detailed methods were not provided by Samitz and Shrager (1966) regarding their skin stripping technique or any of the experiments for the most part. However, these experiments enabled comparison of the Cr(III) compounds with varying physicochemical characteristics (e.g. ionic strength, pH) and skin penetrating capabilities in a subsequent study (Samitz *et al.,* 1967).

Initial results of the 1966 experiment with intact skin indicated one subject developed mild ($^{+}1$) positive reactions to $CrCl_3$ (5%) and $Cr_2(SO_4)_3$ (0.5% and 1%). An explanation of the scoring scale was not provided. However, tests with 0.25% $K_2Cr_2O_7$ produced ($^{+}2$ to $^{+}3$) responses in all five subjects. In stripped-skin tests, 5% $CrCl_3$ produced $^{+}2$ responses in two subjects. These individuals also had $^{+}1$ or $^{+}2$ responses to 5% $Cr(NO_3)_3$. The subject with the stronger response to $Cr(NO_3)_3$ also had $^{+}1/^{+}2$ responses 0.5% and 1% $CrCl_3$. The tested Cr(III) compounds produced only equivocal or mostly negative results in the two subjects with no positive responses. These results were similar to the authors' previously published preliminary work, in which the relative penetrating capabilities were Cr(VI) = $CrCl_3$ > $Cr(NO_3)_3$ > $Cr_2(SO_4)_3$. A later study (Samitz *et al.,* 1967) confirmed the relative penetration potency of Cr(III) in isolated epidermal tissues removed from humans during autopsy. The authors recognized that the skin-stripping process performed in the 1966 study enabled the poorly and slowly diffusing Cr(III) compounds to better penetrate the skin, overcoming their initial inefficacy to become elicitors of hypersensitivity responses.

Though the dermal sensitization studies do not provide usable data for quantitative risk assessment purposes, they do lend insight into the ability of Cr(III) compounds to cause sensitization and elicit sensitization reactions in Cr(III)- and Cr(VI)-sensitized individuals. A later report by Novey *et al.* (1983) provided some additional information as to the mechanisms by which Cr(III) allergenicity is manifested. As a whole, the findings suggested to OEHHA that Cr(III) allergies were caused by immediate (Type 1) and possibly delayed (Type 4) hypersensitivity immune reactions. Type 1 hypersensitivity to Cr(III) was supported by a later report (Park *et al*., 1994) of occupational asthma caused by exposure to $Cr_2(SO_4)_3$ salts.

In Type 1 reactions, contact with an antigen, e.g. inhalation of a Cr(III) compound, causes the formation of type E immunoglobulins (IgE antibodies) that coat mast cells

and basophils circulating in the tissues and blood of the exposed individual. Upon subsequent exposures, the previously formed, cell-bound, antigen-specific IgE antibodies bind to the antigen. This causes the mast cells and basophils to release a mixture of compounds (e.g. histamine and proteases) that trigger rapid allergic responses including but not limited to the contraction of smooth muscles in the airways (bronchospasm), coughing, wheezing, and asthma. These allergic responses begin in the first few minutes of exposure and extend to up days after the subsequent exposure (AMBOSS, 2019).

In Type 4 reactions, contact with an antigen, e.g. dermal penetration of a Cr(VI) compound, causes uptake by Langerhans cells which migrate from the skin of the exposed individual to his/her lymph nodes to form sensitized T-cells. In this example, Cr(VI) would reduce to Cr(III) after penetrating the skin and complex with endogenous carrier molecules (e.g. proteins) to form a hapten, a larger molecule capable of eliciting an immune response, which is recognized as foreign. The hapten is then bound, internalized, processed, and transported by Langerhans cells (Bregnbak *et al.,* 2015).

Because Cr(III) is the form presented to T-cells in this initial exposure, subsequent exposures to Cr(VI) or Cr(III) compounds cause the sensitized T-cells to release cytokines (chemical messengers) that mediate inflammation. Examples include but are not limited to: interferon gamma, which activates macrophages and enhances their phagocytic and killing mechanisms; tumor necrosis factor beta, which activates endothelial cells and enhances vascular permeability; and interleukin 3, which activates mast cells. Inflammatory responses generally develop 12-48 hours after the subsequent exposure (AMBOSS, 2019), with contact dermatitis being a commonly observed pathology.

According to the National Institutes of Health (2018), Cr(III)-related dermatitis is usually seen only with prior sensitization to Cr(VI). This is because the bioavailability of the chromium antigen is essential for sensitization, and Cr(VI) compounds (e.g. dichromates) penetrate the skin more readily the Cr(III) ones. However, sensitization by water-soluble Cr(III) compounds, independent of Cr(VI), cannot be ruled out (Arfsten *et al.*, 1998). This is especially true when skin permeability is increased via physical or chemical means prior to exposure. Asthma caused by delayed hypersensitivity responses is primarily mediated by immune cells (e.g. eosinophils[12]) recruited by mast

[12] An eosinophil is a type of white blood cell (WBC; leukocyte) that is normally found in low numbers in blood relative to other WBCs. In general, eosinophil levels that exceed 5% of the total number of leukocytes in a blood sample are considered elevated, though this cut-off can vary slightly by laboratory (Kovalski and Weller, 2016). Increased numbers of eosinophils in blood can be indicative of allergy, parasitic infection, or cancer.

cells. Eosinophils produce cytokines and proteins that result in bronchoconstriction, airway damage, tissue remodeling, and asthma exacerbation.

Novey et al. (1983)

According to their case report, a 32-year old white male patient, with no pets, personal/family history of allergies, or previous episodes of asthma, lung disease, or tuberculosis exposure, developed a productive cough with clear sputum, wheezing, and dyspnea (difficult, labored breathing) less than 2 weeks after starting a new job electroplating with Cr and Nickel (Ni). Previous work for several years in electroplating factories with exposures to cadmium or gold had not produced similar adverse pulmonary effects. The patient's respiratory distress improved with a 1-week medical leave from his new job, but within 1 hour of exposure upon his return, the wheezing and dyspnea also returned.

The patient was provided with antibiotics and antihistamines (treatment regimen not stated) and assessed via chest x-ray by his physician, but the x-ray was reported "negative," and the patient returned to work against his physician's advice. It is unclear to OEHHA which pathology was determined to be "negative". With his return to work, the patient experienced even more severe dyspnea which peaked 2 days later. Examination by Novey *et al*. occurred 2 days after the peak effects and revealed the patient was "healthy" aside from abnormal lung findings, including sporadic dry cough, expiratory wheezing, inspiratory rales (clicking/rattling sounds), elevated levels of eosinophils in blood, and evidence of obstructive airway disease upon pulmonary function tests (PFTs). In order to test the patient's allergic responses to Cr and Ni salts and determine whether the patient could return to work in the metal-plating industry, Novey *et al*. (1983) performed broncho-provocation, skin challenge, and serologic tests.

After the patient avoided all medication for 24 hours, and prior to double-blind[13] broncho-provocation tests, baseline PFT results were obtained. The patient was subjected to broncho-provocation tests only when his baseline lung mechanics (PFT results) were ≥75% of the predicted value. In these lung challenge tests for allergies, a small amount of the suspected allergen (Cr salt in this case) is inhaled or ingested by the patient so researchers can observe whether it triggers an allergic response (e.g. asthma and a change in PFT results).

[13] In double-blind experiments, neither the test subjects nor the researchers know which subjects are receiving a particular treatment. This information, which may influence subject/researcher behavior, is withheld until after the experiment is completed.

Broncho-provocation tests by Novey *et al*. (1985) were performed with one metal salt or control solution at a time, in 5-minute exposure scenarios that simulated the patient's work exposures. Test Cr(III) sulfate solutions were provided by the patient from his job site, but chemical concentrations and formulas were not stated. The control Cr solution was phenol red dye in 0.01 M acetic acid (vinegar diluted 100-fold) with a few drops of 1% chromic acid [a Cr(VI) compound] added to simulate the odor of the Cr(III) sulfate used in the factory. In each simulated work scenario, the patient painted a 10-inch square zinc mesh with and breathed heat-generated fumes from one of the solutions. Neither occupational nor simulated lung challenge exposures were quantified or chemically analyzed by Novey *et al*. (1985); however, the authors reported that according to the patient, the simulated fume exposures were comparable in degree to those he encountered at work. A total of three simulated exposures were performed for each solution, and after each exposure, PFTs were given to the patient every five minutes for 20 minutes. If no changes in lung mechanics occurred during that time, the patient was challenged with a different solution. If a "positive" response occurred, the PFTs were performed every 15 minutes for 2 hours, then every 30 minutes for 3 hours to allow Novey *et al*. to monitor the patient's reaction. The "positive" response was defined by Novey *et al*. (1983) as a >15% drop in the patient's FEV_1, a measurement of the maximal amount of air he could forcefully exhale in one second, and a marker of the magnitude of his asthmatic airway obstruction.

Broncho-provocation tests with control solutions yielded no changes in PFT results. However, upon the first lung challenge with Cr(III) sulfate, a recurrence of his work-related symptomology was observed within the first 15 minutes PE. Associated changes in lung mechanics included a 22% drop in FEV_1, a 25% drop in peak expiratory flow rate (PEFR), and a 14% drop in his FEV_1:FVC ratio that gradually improved without therapy to near-baseline levels in 90 minutes. PEFR is the maximum speed of expiration, and FVC (forced vital capacity) is the total amount of air that can be forcibly exhaled from the lungs after taking the deepest breath possible. Measurements of the PEFR and FEV_1:FVC ratio can be used to distinguish obstructive lung diseases like asthma from restrictive ones like pulmonary fibrosis. In the case study by Novey *et al*. (1983), Cr(III) sulfate broncho-provocation test results were indicative of the former.

Skin prick tests[14] were then performed on the subject and two "atopic" individual controls with analytical-grade Cr(III) sulfate [$Cr_2(SO_4)_3 \times H_2O$] diluted with phosphate-

[14] Skin prick/puncture/scratch tests can be used to check for immediate (Type 1) allergic reactions (i.e. presence of IgE antibodies) to up 40 different substances at once. During the test, small needles are used to deposit allergens into the surface layer of skin on the subject's forearm or upper back to enable the tester to observe the magnitude of response to each separate allergen. Response magnitude is

buffered saline to 0.1, 1, 5, and 10 mg/mL. No background information was given regarding the two allergic individuals. No immediate or later reactions occurred, but false-negative responses are a known limitation of skin prick tests (MFMER, 2019), and Novey *et al*. acknowledged that their test concentrations were conservatively low to prevent robust systemic reactions.

Therefore, serological radioimmunosorbent assays and radioallergosorbent tests (RASTs)[15] were performed to identify total and antigen-specific serum IgE antibodies, respectively, in duplicate serum samples from the subject and 10 atopic control individuals (50 µL each). RAST antigens included $Cr_2(SO_4)_3 \times H_2O$, gold (sodium aurothiomalate), and 10 unspecified "common, indigenous allergens." The atopic individuals had suspected allergic bronchopulmonary diseases but no known exposure to metal plating. The subject's total serum IgE level was within normal limits. His average RAST score was more than 3 times that of the controls for Cr(III), but not different (statistical methods not stated) from controls for gold, and negative for the 10 common allergens. Overall, results indicated to Novey *et al*. that the subject was not an atopic person in general but was allergic to Cr(III) fumes, specifically, and his responses were mediated by Type 1 mechanisms. Given the temporal patterns of the subject's adverse responses to Cr(III), i.e. asthmatic within minutes of exposure but normal otherwise, the increasing severity and rapidity of responses with subsequent occupational exposures, and the results of RAST and challenge tests, OEHHA agrees this is likely the case.

The tests with Ni compounds are mostly not discussed herein, but the patient did exhibit 1) an acute drop in spirometric values and exacerbation of symptoms (chest tightness, wheezing) upon inhaling fumes from a nickel sulfate solution versus a control solution; 2) spontaneous resolution and recurrence of these symptoms within 2 and 5 hours PE, respectively; 3) a negative skin prick test; and 4) a positive RAST test with elevated serum levels of Ni-specific IgE antibodies relative to control subjects. The results indicated to Novey *et al.* (1983) that the patient's responses to Ni were mediated at least in part by a Type 1 allergic reaction.

measured by the diameters of the weal (a raised itchy bump), and the surrounding flare (area of redness) that develop in the ~15 minutes following the prick.

[15] RASTs involve the addition of antigen, bound to an insoluble material, to a blood serum sample collected usually from a subject's arm. Antigen-specific IgE antibodies can be quantified by the subsequent addition of radiolabeled antibodies that bind to them. As unbound radiolabeled antibodies are washed away, the amount of radioactivity in a serum sample is proportional to the number of IgE antibodies bound specifically to the antigen.

Park et al. (1994)

Similar to Novey *et al.* (1983), Park *et al.* performed broncho-provocation, skin challenge, and PFTs in their examinations of 4 males with occupational asthma resulting from work-place exposure to Cr. Minimal details were provided regarding the workplace exposures and study materials and methods.

The subjects were ex-smokers ranging in age from 26-54 years and working in metal plating (n = 2; Subjects A & B), cement (Subject C), or construction industries (Subject D). It is unknown to OEHHA whether the Cr(III) or Cr(VI) species caused the subjects' occupational asthma, but Cr(VI) sensitization is known to occur in these occupations. All of the subjects complained of asthmatic symptoms during and after work hours, but asthma latency in the subjects ranged from 3 to 108 months. Some reported associated symptoms like rhinitis (Subjects B & D) or urticaria (Subject A). None had contact dermatitis.

Park *et al.* characterized Subjects A, B, and D as having atopy. Atopy was defined as a positive response score of $>2^{+}$ for ≥2 of 50 unstated "common inhalant allergens" included in their skin prick tests. These scores seemed to OEHHA to be obtained by measuring the mean maximum orthogonal diameters of the weal (swollen area) and erythema (patchy skin redness) resulting 15 minutes after a skin prick with a specific allergen, and dividing the weal diameters by those of the erythema[16]. Skin prick tests performed with 10 mg/mL trivalent $Cr_2(SO_4)_3$ revealed two subjects (B & C) with immediate positive test results. These two subjects had negative skin patch tests performed with 0.5% hexavalent $K_2Cr_2O_7$ and read 48 hours post application. Response severity was not reported for the 2 subjects (A & D) with positive patch test results.

PEFR monitoring was done every 2 hours for 2 consecutive days in the two subjects (A & B) working metal-plating jobs. PEFR was "significantly decreased" during and after work, with dyspnea and/or urticaria reported 2-7 hours after work. The subjects were advised to discontinue chromium exposure and take asthma medication.

Methacholine broncho-provocation tests[17] were performed to evaluate the reactivity of each subject's lungs. In these tests, an aerosol of 0-9% NaCl followed by serial doubling

[16] In a case where the orthogonal, maximum weal diameters are A and B, and those of the erythema are Y and Z, the skin prick score = (A × B) ÷ (Y × Z).

[17] Methacholine is a drug that causes narrowing of the airways similar to what is seen with asthma. Methacholine challenge tests begin with baseline breathing tests to determine lung function, including FEV_1, prior to administration of drugs/medications. Afterward, progressively larger doses of methacholine are inhaled by the test subject, with lung function tests performed before and after every dose to measure changes in airway narrowing. The test stops once FEV_1 drops by ≥20% from baseline, indicating a

concentrations of methacholine (0.75-25 mg/mL), were given by inhalation. FEV_1 measurements were taken 3 minutes after the start of each new exposure and plotted on a response curve to determine the PC_{20}, the methacholine provocation concentration causing a 20% fall in FEV_1. Airway hyperresponsiveness was considered by Park *et al.* to be present if a >20% change in FEV_1 was observed at any concentration in the tested range. The order of airway hyperresponsiveness was such that Subject D > C > B > A, with PC_{20} values of 0.1 mg/mL, 0.5 mg/mL, 4 mg/mL, and > 25 mg/mL, respectively.

Chromium broncho-provocation tests were performed in a laboratory over 8 hours. A sham challenge, in which normal saline was inhaled, was performed on a day prior to the actual tests with $Cr_2(SO_4)_3$. For these latter tests, 0.1, 1, and 1 mg/mL solutions were made with normal saline and the Cr(III) salt, and nebulized for inhalation. During the test period, the concentration of the nebulized material was increased in 10 minute intervals, and subjects were asked to breathe each test aerosol from functional residual capacity to total lung capacity for five breaths until a ≥20% drop in FEV_1 was observed. Functional residual capacity is the volume of air in the lungs at the end of a normal expiration. Total lung capacity is the volume of air in the lungs at the end of a maximal inspiration. FEV_1 and MMEF (maximum midexpiratory flow) were measured by spirometry every 10 minutes during the first hour, and hourly thereafter for 8 hours. A bronchodilator was inhaled and oral theophylline and steroids were administered when the subjects had severe asthmatic responses.

According to Park *et al.*, two “healthy controls” and two “intrinsic asthma patients” showed negative responses to the $Cr_2(SO_4)_3$ broncho-provocation test up to 10 mg/mL, but no additional information was provided regarding these individuals. All four of the test subjects with occupational asthma had clear responses to the $Cr_2(SO_4)_3$ aerosols, with the maximum FEV_1 decline ranging from approximately 45% to nearly 70%. Subject A exhibited an early and severe asthmatic response that began after exposure to the 0.1 mg/mL concentration and nearly resolved by the end of the test period (Figure 4A).

Though Subject A previously had a negative methacholine test result (PC_{20} >25 mg/mL), follow-up tests revealed airway hyperresponsiveness and resolution at 24 hours and 3 days after the $Cr_2(SO_4)_3$ challenge test, respectively. The follow-up methacholine test results suggested to the study authors that Subject A developed airway hyperreactivity after the isolated, early asthmatic reaction to the $Cr_2(SO_4)_3$

positive test result, or the maximum dose of methacholine is reached without a change in lung function, indicating a negative result. The latter nearly rules out an asthma diagnosis. Bronchodilating medications are provided once the test is complete or the subject develops discomfort, and breathing tests are repeated to ensure the subject’s lungs return to normal (AAAAI, 2019).

challenge. In contrast, Subjects B-C had dual responses in their $Cr_2(SO_4)_3$ provocation tests, with recurrent FEV_1 declines interspersed by periods of partial recovery (Figures 4B-D).

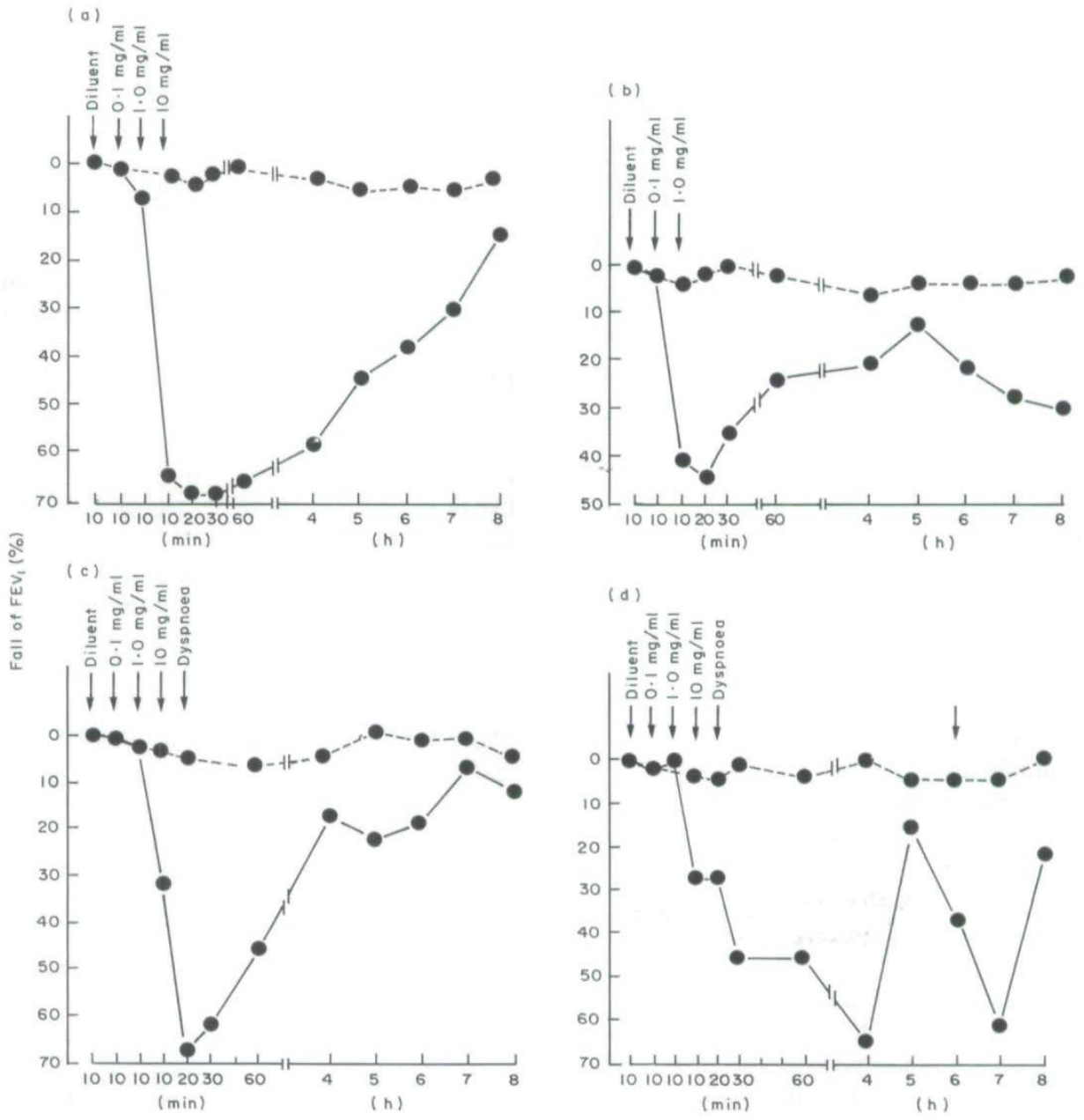

Figure 4. Results of bronchoprovocation testing with $Cr_2(SO_4)_3$ in study subjects. The figure was copied from Figure 1 of Park *et al.* (1994). Dashed and solid lines indicate sham and trivalent $Cr_2(SO_4)_3$ challenge results, respectively. Abbreviations: h = hours; min = minutes.

After a period 3-22 months, follow-up exams showed that Subjects B-D were avoiding Cr exposures. Subjects B and C were taking sodium cromoglycate as an asthma preventative medication. Subject B paired this with a bronchodilator (an asthma rescue medication). Subject D was the least sensitive to methacholine challenge (PC_{20} >25

mg/mL). Results for Subject B were 0.62 mg/mL, slightly different than they had been, and those for Subject C were decidedly worse (14 mg/mL). Patient A was lost to follow-up. Overall, the results by Park *et al*. (1994) suggested to OEHHA that inhalation of a Cr(III) compound may result in an asthmatic response in individuals previously shown to be dermally sensitized to Cr(III) or Cr(VI) compounds, and bolstered findings of a Type 1-mediated response by Novey *et al.* (1983).

According to the US Agency for Toxic Substances Disease Registry (ATSDR, 2012), while chromium-induced asthma may occur in some sensitized individuals exposed to elevated concentrations of chromium in air, the number of sensitized individuals is low, and the number of potentially confounding variables [e.g. exposure to other allergenic metals] in the chromium industry is high. They indicate prevalence of chromium sensitivity in the general population of the US ranges from 0.08% - 7% depending upon the subpopulation evaluated (ATSDR, 2012). However, the original source of the range was not provided, and it was initially unclear to OEHHA whether the statement pertained to Cr(III), Cr(VI), or all chromium species. OEHHA found the stated range likely came from several skin patch studies testing allergies to Cr(VI) compounds. These studies are summarized below.

Proctor et al. (1998)

OEHHA believes the lower-bound estimate of 0.08% was calculated by Proctor *et al.* (1998), who reviewed skin patch studies from 1950-1996 to summarize previously reported prevalence rates of Cr(VI) allergy ranging from 2 – 8% in clinical populations from North America and 0 - 19.5% in general, clinical, and/or occupational populations from Europe. Skin patch tests are used to diagnose Type 4 hypersensitivity reactions. Proctor *et al*. also used data from the North American Contact Dermatitis Group (NACDG) to determine the prevalence of Cr(VI) allergy in a clinical cohort from the US and two studies from the Netherlands (Lantinga *et al*., 1984; van Ketel, 1984) to determine an approximate ratio of prevalence rates in clinical versus general populations.

According to Proctor *et al.*, the NACDG 1) standardized diagnostic skin patch testing procedures and scoring criteria to minimize non-allergic irritant responses to test substances, 2) noted the relevance of positive test results, and 3) used physician NACDG members, experts in diagnosing contact allergy, to determine the prevalence of Cr(VI) allergy from 1992-1996. The NACDG's clinical cohort consisted of 6515 patients suspected of having allergic contact dermatitis. Of the 131 patients with positive responses to a Cr(VI) skin patch test, 68 (52%) were determined by the NACDG to be "relevant" (i.e. supported by historical dermal exposure to the putative allergen), and half these (n = 34) were classified as occupationally related. Using only results

determined to be “relevant”, the prevalence of Cr(VI) allergy in the NACDG cohort was calculated at approximately 1% (68 ÷ 6515 = 0.01). To estimate a general prevalence rate for the US, Proctor *et al.* divided the clinical prevalence in the US (1%) by 12, the approximate ratio of the prevalence rates in a clinical dermatology patient population (5.8%; n = 105 of 1776; van Ketel, 1984) and the adult general population (0.5%; n = 9 of 1992; Lantinga *et al*., 1984) of the Netherlands. The researchers calculated an estimate of 0.08% (1% ÷ 12 × 100 = 0.08%).

Weston et al. (1986)

OEHHA found one study (Weston *et al*.; 1986) reporting chromium allergy prevalence in the US at a proportion of 7.6%, similar to the upper-bound estimate (7%) given by ATSDR (2012). The study by Weston *et al.* examined 314 “healthy” children (166 boys, 148 girls), age ≤18 years, for skin patch test responses to 20 different substances including hexavalent $K_2Cr_2O_7$ (0.5% in petrolatum). Volunteer subjects were recruited from the Denver, CO metropolitan area, and divided into three groups by age (6 months – 5 years, 5 – 12 years, and 12 – 18 years). There were 264 “white,” 41 “black,” and 9 “Oriental” children representing 84%, 13%, and 3% of the study population, respectively, with 129 (41%) in the youngest, 113 (36%) in the middle, and 71 (23%) in the oldest age groups.

The test substances were recognized by the NACDG and the Task Force on Contact Dermatitis of the American Academy of Dermatology to be frequent causes of allergic contact dermatitis. Each child was dermally exposed to all 20 substances for 48 hours via Finn chambers affixed to a hypoallergenic tape and applied to a section of normal (no redness or papules), alcohol-cleansed skin on the back. Each Finn chamber held a 20-µL volume of a single test substance. Examinations occurred one day after the chambers were removed, 72 hours after the start of the exposure. Severity of skin responses was scored on a semi-quantitative ordinal scale that distinguished irritant from allergic reactions. Scoring was performed by one individual and verified by a second observer.

There were 24 children with positive reactions to hexavalent $K_2Cr_2O_7$, the same number with positive reactions to nickel sulfate (2.5% in petrolatum). These two chemicals, along with neomycin sulfate (an antibacterial agent), accounted for most of the total positive reactions, with 7.6% (n = 24/314) prevalence for $K_2Cr_2O_7$ and nickel sulfate allergy, and 8.1% (25/314) for neomycin sulfate allergy. The source of chromium sensitization was assumed by the authors to be leather athletic shoes, consistent with previous studies on foot dermatitis and suspected contact dermatitis in children <12 years of age. The authors reported “no significant racial or sex differences” in skin patch test results. However, age-, race-, and sex-specific data were aggregated for the group

of tested chemicals, so it is mostly unknown to OEHHA how the prevalence and severity of $K_2Cr_2O_7$ allergy differed by these parameters.

Allergy prevalence was <4% for each of the other tested chemicals. Transient irritant reactions to test substances were observed in 21 of the 314 subjects (11 boys, 10 girls), with none of the test substances predominating in the number of irritant responses. Irritant responses to the application tape were also observed in 26 subjects (9 boys, 17 girls), with the reactions occurring at the margins of the tape, distant from the Finn chambers.

OEHHA found three other patch test studies performed in children; however, these studies were conducted in Europe with individuals suspected of having contact dermatitis. The prevalence of Cr(VI) allergy was approximately 5% for all three studies: 6 of 125 Scottish children <12 years of age (Rademaker and Forsyth, 1989), 9 of 168 Danish children ≤14 years of age (Veien *et al.*, 1982), 17 of 349 Polish children age 3 - 14 years and 34 of 626 Polish children age 3 - 16 years (Rudzki and Rebandel;1996).

Though the prevalence estimates were determined using data from subjects sensitized to Cr(VI) compounds, Cr(III) sensitivity is recognized by the US National Institute of Health to occur after sensitization to Cr(VI) compounds. There are several human and animal studies that have shown Cr(III) or Cr(VI) cross-reactivity after sensitization with one of the two species. Animal studies are discussed in Section 5.2, below.

OEHHA understands that Cr(VI) compounds generally have a lower threshold dose than Cr(III) compounds with respect to eliciting allergic dermatitis responses. Given skin patch tests are used to determine non-specific delayed-type hypersensitivity reactions in which the allergenic component is ultimately a Cr(III) hapten (Bregnbak *et al.,* 2015), and Cr(III)↔Cr(VI) cross-reactivity has been shown to occur in sensitized animals (Table 6), the prevalence range reported by ATSDR for Cr(VI) allergy in the US were used by OEHHA, in the absence of Cr(III)-specific data, as rough worse-case estimates of Cr(III) allergy prevalence in CA.

A prevalence of 0.08% - 7% would account for approximately 316,456 – 2,768,993 Californians based upon the most recent California population estimate of 39,557,045 from the US Census Bureau (USCB, 2018). This assumes an equal distribution of Cr-sensitized individuals in the US and California.

5.2 Cr(III)/Cr(VI) Cross-reactivity Studies in Guinea Pigs

One of the most comprehensive tests of Cr(III)/Cr(VI) cross-reactivity was performed by Gross *et al*. (1968). They performed experiments to test these outcomes in albino guinea pigs sensitized and challenged with different Cr compounds. Sensitization was performed with a total of three subcutaneous injections (SCIs) in the nape of the neck performed one week apart. The injectants were emulsions of 0.5 cc Freund's complete adjuvant[18] with either 0.5 cc of hexavalent $K_2Cr_2O_7$ (3.4×10^{-3} M; n =27) or trivalent $CrCl_3 \times 6H_2O$ (3.4×10^{-2} M; n = 13), except for the control animals which received Freund's adjuvant alone during sensitization. According to the authors, ulceration was observed frequently at the injection site for Cr(VI)- and Cr(III)-, but not control-exposed guinea pigs. The ulcers were said to be the result of irritation, but they invariably healed in 2-3 weeks.

Initial allergen challenge experiments were performed three weeks post-sensitization (PS) with a single 0.1-cc SCI of $K_2Cr_2O_7$ or $CrCl_3 \times 6H_2O$ (4.2×10^{-4} M) in physiologic saline. Examinations were performed 48 hours after challenge. The authors noted that sensitization occurred irrespective of previous ulceration during the sensitization period. Briefly, 26/27 animals developed positive skin responses when given $K_2Cr_2O_7$ as a sensitization and challenge chemical. Positive skin tests, indicative of $K_2Cr_2O_7$ sensitization, were determined by the presence of an indurated (hardened, thickened) erythematous papule ≥10 mm in diameter ($^+1$). Of the 26 with positive responses, skin reactions >15-20 mm in diameter ($^+2$; n = 11), >20 mm in diameter ($^+3$; n = 11); and containing central necrosis ($^+4$; n = 1) were also observed. When $CrCl_3 \times 6H_2O$ was given as the sensitization and challenge chemical, 10/13 had positive skin responses indicative of Cr(III) sensitization. Response severity ranged from $^+1$ (n = 6) to $^+2$ (n =4).

Cross reactivity experiments indicated a significant (p = 0.005) difference in response to $K_2Cr_2O_7$ versus $CrCl_3 \times 6H_2O$ challenge in $K_2Cr_2O_7$-sensitized animals, as they exhibited more severe responses to the Cr(VI) challenge. However, when a similar experiment was performed in $CrCl_3 \times 6H_2O$-sensitized guinea pigs, the majority of reactions were similar among those challenged with $K_2Cr_2O_7$ versus $CrCl_3 \times 6H_2O$.

[18] An adjuvant is a substance that boosts the immune response to an antigen. Freund's complete adjuvant is composed of inactivated and dried mycobacteria and effective in stimulating cell-mediated (i.e. phagocytes, T-cells, and cytokine) immune responses.

Additional challenge experiments were performed in $K_2Cr_2O_7$- and $CrCl_3 \times 6H_2O$-sensitized guinea pigs (n = 3/group) given a single 0.1-cc SCI of one of the following Cr(III) salts.

1. chromic acetate (no formula given; 2.5×10^{-3} M)
2. chromic nitrate nonahydrate [$Cr(NO_3)_3 \times 9H_2O$; 9.6×10^{-4})
3. chromic oxalate (no formula given; 2.5×10^{-4} M)
4. chromic sulfate pentadecahydrate [$Cr_2(SO_4)_3 \times 15H_2O$; 2.4×10^{-4} M] salts

While *Gross et al.* did not state the amount of time between each of the challenge experiments with these additional Cr(III) salts, Cr(VI) cross-reactivity was observed as shown in Table 6.

The animals in the study by Gross *et al.* were said to have retained their sensitization when followed for a year, but no associated data were presented. Though the authors performed other experiments with protein-complexed $K_2Cr_2O_7$ and $CrCl_3$ conjugates as sensitization and challenge chemicals, the experiments were largely unsuccessful and are not summarized by OEHHA.

Table 6. Summary of subacute Cr(III)/Cr(VI) cross-reactivity studies in guinea pigs.

Reference	Sensitization + Challenge	Results
Gross *et al*. (1968)	$K_2Cr_2O_7$ + $K_2Cr_2O_7$	N = 26/27 sensitized; scores ranged +1 to +4 (inflammation and swelling to focal necrosis)
As above	$K_2Cr_2O_7$ + $CrCl_3 \times 6H_2O$	N = 26/26 cross-sensitized. In comparison to $K_2Cr_2O_7$ challenge, rxn severity was ↓ (n= 17), equal (n = 8), or ↑ (n = 1).
As above	$K_2Cr_2O_7$ + chromic acetate	N = 3/3 cross-sensitized. In comparison to $K_2Cr_2O_7$ challenge, rxn severity was ↓ (n= 2) or equal (n = 1).
As above	$K_2Cr_2O_7$ + $Cr(NO_3)_3 \times 9H_2O$	N = 3/3 cross-sensitized. In comparison to $K_2Cr_2O_7$ challenge, rxn severity was equal (n = 3).
As above	$K_2Cr_2O_7$ + $Cr_2(SO_4)_3 \times 15H_2O$	N = 3/3 cross-sensitized. In comparison to $K_2Cr_2O_7$ challenge, rxn severity was equal (n = 2) or ↓ (n= 1).
As above	$K_2Cr_2O_7$ + chromic oxalate	N = 2/3 equivocal response. N = 1/3 no response.

Abbreviations: ↑ – increased; ↓ – decreased; $CrCl_3$ – chromium (III) chloride; $CrCl_3 \times 6H_2O$ – chromium (III) chloride hexahydrate; Cr(III) – trivalent chromium; $Cr(NO_3)_3 \times 9H_2O$ – chromium (III) nitrate nonahydrate; $Cr_2(SO_4)_3 \times 15H_2O$ – chromium (III) sulfate pentadecahydrate Cr(VI) hexavalent chromium; $K_2Cr_2O_7$ – potassium dichromate, a Cr(VI) chemical; rxn – reaction.

Table 6. Summary of subacute Cr(III)/Cr(VI) cross-reactivity studies in guinea pigs (continued).

Reference	Sensitization + Challenge	Results
Gross *et al*. (1968)	$CrCl_3 \times 6H_2O$ + $CrCl_3 \times 6H_2O$ 10/13 sensitized. 4/13 had +2. No +3 or +4 rxns.	N = 10/13 sensitized; scores ranged +1 to +2 (inflammation up to 20 mm in diameter)
As above	$CrCl_3 \times 6H_2O$ + $K_2Cr_2O_7$	N = 8/10 cross-sensitized. In comparison to $CrCl_3 \times 6H_2O$ challenge, rxn severity was equal (n = 5), ↓ (n = 2), or ↑ (n = 1).
As above	$CrCl_3 \times 6H_2O$ + chromic acetate	N = 2/3 sensitized. In comparison to $CrCl_3 \times 6H_2O$ challenge, rxn severity was equal (n = 1) or ↓ (n = 1)
As above	$CrCl_3 \times 6H_2O$ + $Cr(NO_3)_3 \times 9H_2O$	N = 3/3 sensitized. In comparison to $CrCl_3 \times 6H_2O$ challenge, rxn severity was equal (n = 2) or ↓ (n = 1)
As above	$CrCl_3 \times 6H_2O$ + $Cr_2(SO_4)_3 \times 15H_2O$	N = 3/3 sensitized. In comparison to $CrCl_3 \times 6H_2O$ challenge, rxn severity was ↓ (n = 2) or equal (n = 1)
As above	$CrCl_3 \times 6H_2O$ + chromic oxalate	N = 2/3 equivocal response. N = 1/3 no response.

Abbreviations: ↑ – increased; ↓ – decreased; $CrCl_3$ – chromium (III) chloride; $CrCl_3 \times 6H_2O$ – chromium (III) chloride hexahydrate; Cr(III) – trivalent chromium; $Cr(NO_3)_3 \times 9H_2O$ – chromium (III) nitrate nonahydrate; $Cr_2(SO_4)_3 \times 15H_2O$ – chromium (III) sulfate pentadecahydrate Cr(VI) hexavalent chromium; $K_2Cr_2O_7$ – potassium dichromate, a Cr(VI) chemical; rxn – reaction.

5.3 Other Toxicity Studies in Rodents and Rabbits

Acute exposure studies in rodents indicated that inhalation of water-soluble Cr(III) compounds at concentrations ≥2.8 mg/m^3 (2800 µg/m^3) may produce inflammation and cell membrane damage in the lungs and initiate edematous buildup in alveolar capillaries. However, some of these effects may have been related to the acidity of the tested Cr(III) salt. Insoluble Cr(III) produced dose-dependent levels of Cr(III)-laden macrophages, but no other statistically significant ($p \leq 0.05$) effects at concentrations as high as 44 mg/m^3 (44,000 µg/m^3).

Henderson et al. (1979)

After exposure to a nebulized trivalent $^{51}CrCl_3$ x $6H_2O_3$ aerosol at concentrations of 0, 2.8, or 77 mg/m^3 (0, 2800, or 77,000 µg/m^3) for 30 minutes, Syrian hamsters of unstated age were sacrificed at 2 hours or 1, 7, or 21 days PE. There were 4 hamsters/sex/treatment group/time-point. Upon necropsy, lung histopathology was assessed, and radioactivity, biochemical variables, and nucleated cells in lung tissue homogenate and/or BALF were quantified. Biochemical variables included the intracellular enzymes[19], lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (glu-6P-DH); the plasma membrane-associated enzyme, alkaline phosphatase (ALP); acid phosphatase (AP); beta (β)-glucuronidase, a lysosomal enzyme; soluble collagen; and trypsin inhibitory capacity – all indicators of cellular injury when elevated in lung tissues and/or BALF[20].

Hamsters exposed at 2.8 mg/m^3 (low-exposure) or 77 mg/m^3 (high exposure) were reported to have initial lung burdens of 0.71 ± 19 µg (0.00071 ± 0.019 mg) or 20.4 ± 9.7 µg (0.0204 ± 0.0097 mg) radiolabeled Cr, respectively, at 2 hours PE (Table 4). Microscopic examinations of the lungs of all Cr-exposed hamsters sacrificed 1 day PE revealed mostly “normal” tissue with focal accumulations of macrophages and polymorphonuclear leukocytes (PMNs, e.g. neutrophils, eosinophils). These cells were present in alveoli adjacent to respiratory and terminal bronchioles with diffuse congestion in alveolar capillaries, but no morphological damage.

These changes were not reflected in BALF cell differentials but were considered by Henderson *et al*. to be representative of mild, nonspecific irritation. The histopathology reported at 2.8 mg/m^3 would be consistent with a severity level of 0-1 according to OEHHA’s (2008) TSD for non-cancer RELs. A score of 0 indicates no observed effects, and a score of 1 indicates enzyme induction or other biochemical changes (excluding signal transduction effects) consistent with possible mechanism of action, but no pathologic changes, no change in organ weights, and no downstream adverse developmental effects (OEHHA, 2008).

[19] As LDH and glu-6P-DH are intracellular enzymes, the presence of one or both in the extracellular space can serve as an indicator of disturbances to cellular integrity (e.g. cell membrane damage that occurs with necrotic cell death).

[20] Alkaline phosphatase is a general marker of lung tissue damage and alveolar Type II cell proliferation (Capelli et al., 1997). It has also been shown to control chemotaxis of inflammatory polymorphonuclear cells (PMNs; Corriden et al., 2008; Junger, 2008; Li et al., 2016). PMNs are recruited to sites of damage, inflammation, or infection as mediators of the immune response. PMNs and macrophages release acid phosphatase and β glucuronidase during phagocytosis, upon damage to their own cell membranes, or with death by necrosis (Henderson et al., 1979).

No statistically significant ($p < 0.05$) differences were observed in lung homogenate or BALF biochemistry between the low-exposure and control groups. Thus, the 2.8 mg/m^3 exposure concentration was considered by OEHHA to be the no observed adverse effect level (NOAEL) for all examined time-points. Comparisons of lung homogenates from high-exposure hamsters and controls revealed that in the high-exposure hamsters, there were: 1) a 75% increase ($p < 0.05$) in AP activity at 1 day PE with resolution to near-control levels on days 7 and 21 PE; 2) an increase of unstated magnitude in β-glucuronidase activity at day 1 PE; and 3) a doubling of ALP activity at day 21 PE. Similar comparisons of BALF data showed significantly ($p < 0.05$) increased AP activity at days 1, 7, and 21 PE. BALF ALP activity was low compared to controls at day 1 PE, but high compared to controls at day 2 PE. Quantitative comparisons were not provided by the authors. No other significant differences in measured biochemical parameters were observed relative to controls. The variable BALF ALP activity – low on day 1 PE and high on day 2 PE – was explained by Henderson *et al*. as possibly the result of inhibitory action by Cr(III) [which likely ceased by day 7 PE].

ALP is a marker of lung tissue damage and alveolar Type II cell[21] proliferation (Capelli *et al.*, 1997), and has been shown to control chemotaxis of PMNs migrating toward chemoattractants (Corriden *et al.*, 2008; Junger, 2008; Li *et al.*, 2016). Alveolar Type II cells secrete pulmonary surfactant essential for proper lung function, and proliferate when alveolar tissues are damaged. PMNs are recruited to sites of damage, inflammation, or infection as mediators of the immune response. Along with macrophages, PMNs release AP and β-glucuronidase during phagocytosis and upon damage to their own cell membranes or death by necrosis (Henderson *et al*., 1979).

ALP, AP, and β-glucuronidase are not limited to the alveolar region of the lungs, and lung homogenate data do not allow for conclusions to be made regarding site-specific processes. However, cumulative findings reported by Henderson *et al*. (1979), suggested to OEHHA that the 30-minute inhalation exposure to $^{51}CrCl_3$ at 77 mg/m^3 (77,000 µg/m^3) was sufficient to produce mild but persistent inflammatory responses in the lungs, likely in the gas exchange region, up to 21 days PE.

Johansson and Camner (1986)

In the study by Johansson and Camner (1986), male rabbits (2-3 kg; unstated age, strain, and number) were exposed to water-soluble Cr(III) nitrate [$Cr(NO_3)_3$] at 0.6 mg/m^3 (600 µg/m^3), for one month (6 hours/day, 5 days/week), by inhalation. Following exposure, right lung lobes were lavaged for analysis of morphological and

[21] Type II cells are the progenitor cells of the alveolar epithelium, and they secrete surfactant necessary for proper lung function.

functional changes to macrophages. The macrophages were examined by light and electron microscopy for pathological changes, and tested for phospholipid content. No specific information was provided regarding the exposure system, control animals, or chemical purity. It is unclear to OEHHA whether the exposures were conducted in whole-body (WB) chambers or nose-only tubes.

Results showed that phospholipid content was unchanged, However, there were alveolar Type II cells with increased volume density, and nodular accumulations of alveolar macrophages present in the lungs after the $Cr(NO_3)_3$ exposure period. Macrophages exhibited enlarged lysosomes containing Cr (identified by x-ray microanalysis), and laminated structures similar to the surfactant-secreting lamellar bodies of Type II cells. These results were supported by findings of increased metabolic activity and decreased phagocytic capacity in another study (Johansson *et al.,* 1986b; Section 6.2). The authors stated that the concomitant increases in laminated structures, lysosomes, and phagocytic impairment in macrophages may be due to a reduced capacity to catabolize surfactant.

Although lung surfactant is necessary for normal lung function, too much surfactant can hinder gas exchange. Alveolar macrophages play a significant role in the homeostatic balance of lung surfactant levels. In mice, macrophages have been shown to contribute to half of the surfactant catabolism in the lungs (Ikegami, 2006). In rats, temporary depletion of alveolar macrophages led to an 8-10-fold increase in the surfactant pool size. In humans, impaired surfactant catabolism by macrophages resulted in surfactant accumulation [alveolar lipoproteinosis], edema, and respiratory failure in some patients (Chroneos *et al*. 2009).

Although it appears to OEHHA that the $Cr(NO_3)_3$ exposure in Johansson and Camner (1986) was insufficient to completely overcome the homeostatic mechanisms controlling surfactant levels, as evinced by the unchanged phospholipid content of the lungs, it was sufficient to produce adverse functional decrements in macrophages. Accordingly, the 0.6 mg/m^3 (600 µg/m^3) concentration – a Cr(III) equivalent concentration of 0.13 mg/m^3 (130 mg/m^3) is considered by OEHHA to be a free-standing LOAEL (lowest observable adverse effect level). OEHHA's confidence in the study findings is moderated by the limited methodological information provided by Johansson and Camner (1986). However, similar results and conclusions were reported by Johansson *et al.* in a separate, more detailed publication (1986a; Section 6.2).

Derelanko et al. (1999)

Chromium (III) oxide (Cr_2O_3; CAS 1308-38-9) and basic Cr(III) sulfate [$Cr_2(OH)_x(SO_4)_y$ $NaSO_4$ $2H_2O$); CAS 12336-95-7] toxicity data were reported by Derelanko *et al.* (1999)

in a comparison of water-insoluble and water-soluble Cr(III) compounds, respectively. In their study, 7-week old inbred CDF® (Fischer 344)/Crl BR VAF/Plus® rats (n = 4-5/sex/group) were exposed nose-only to Cr_2O_3 at 4.4, 15, or 44 mg/m^3, basic Cr(III) sulfate at 17, 54, or 168 mg/m^3, or air (control) for 1 or 13 weeks (6 hrs/day, 5 days/week). Cr(III) equivalent concentrations[22] for both Cr(III) chemicals were calculated by the study authors at 3, 10, or 30 mg/m^3. One-week experiments are discussed immediately below, and the 13-week experiment is discussed in Section 6.2, Sub-chronic Toxicity in Animals.

With respect to the one-week studies, it is unclear to OEHHA how much time elapsed between the final exposure and the necropsy. Quantification of BALF components via total cell counts, cell differentials, and spectrophotometric analysis of total and specific protein levels in supernatant revealed significant ($p < 0.05$) changes in cell parameters due to basic Cr(III) sulfate but not Cr_2O_3. Analyzed proteins included β-glucuronidase, LDH, and glutathione reductase[23]. Male and female rats exposed to Cr(III) sulfate exhibited significantly ($p < 0.05$) decreased numbers of total cells in BALF at all tested concentrations in comparison to controls. A corresponding downward trend in the percentage of mononuclear cells and upward trends in the percentages of neutrophils, total protein, and LDH were evident in males and females. However, of these, the only significant ($p < 0.05$) results were decreased mononuclear cells and increased neutrophils in males exposed to the highest concentration (168 mg/m^3) of basic Cr(III) sulfate versus control.

Though the authors acknowledged differences in the concentration ranges of the two tested Cr(III) dusts, they pointed to the lack of changes in Cr_2O_3-exposed rat BALF parameters at a time when crystalline Cr_2O_3 was highly visible in the lung tissue sections by microscopy. Noting similar results in 13-week studies (NTP, 1996a; b), in which inflammatory lesions and increased particle clearance were noted upon exposure to soluble nickel sulfate and persistent non-inflammatory pigment was noted in the respiratory tract of rodents exposed to insoluble nickel oxide, Derelanko *et al.* (1999) suggested that the differential toxicities of basic Cr(III) sulfate and Cr_2O_3 were likely due to differences in physicochemical characteristics (e.g. acidity and water solubility) that influence deposition, tissue responses, and clearance.

Acute and subacute exposure studies in rodents are summarized in Tables 7 and 8 below.

[22] A Cr(III) equivalent dose, is the amount of Cr(III) in a known dose of a specific Cr(III) chemical. Cr(III) equivalent doses are calculated to ensure the administered amount of Cr(III) is the same in toxicological studies comparing the effects of different Cr(III) compounds

[23] Glutathione reductase is an intracellular enzyme that helps protect the lungs from injury by ROS.

Table 7. Summary of acute Cr(III) inhalation studies in rodents.

Reference	Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Henderson *et al*. (1979)	Male & female Syrian hamsters; age not stated; n = 5/sex/group. Nose-only inhalation of $^{51}CrCl_3 \times 6H_2O$ at 0, 2.8, or 77 mg/m^3 for 30 minutes. Necropsy 2 hours, or 1, 7, or 21 days PE.	2.8 mg/m^3: No significant ($p \leq 0.05$) BALF or lung tissue differences. Mostly normal lungs with non-specific inflammation. 77 mg/m^3: In lung homogenate, ↑ β-glucuronidase and AP activity at 1 day PE, ↑ ALP activity at 21 days PE. In BALF, ↑ AP on days 1, 7, and 21 PE, AP variable.	NOAEL = 2.8 mg/m^3 for lung tissue endpoints.

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; ALP – alkaline phosphatase; BALF – bronchoalveolar lavage fluid; Cr(III) – trivalent chromium; LOAEL – lowest observable adverse effect level; NOAEL – no observable adverse effect level; PE – post exposure; WB – whole body.

(a) According to review by OEHHA

Table 8. Summary of subacute Cr(III) inhalation studies in rodents.

Reference	Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Johansson and Camner (1986)	Male rabbits (2-3 kg; unstated age, strain, and number). Inhalation exposure to $Cr(NO_3)_3$ at 0.6 mg/m^3 for 1 month (6 hrs/day, 5 days/wk).	↑ metabolic activity and ↓ phagocytic capacity in macrophages[c]	LOAEL[b] = 0.6 mg/m^3 for adverse functional decrements in macrophages
Derelanko *et al.* (1999)	Male & female rats; age 7 wks; n = 5/sex/group. Nose-only inhalation of chromic oxide dust at 0, 4.4, 15, or 44 mg/m^3 for 1 week (6 hrs/day, 5 days/week). Cr(III) equivalent concentrations[a] were 0, 3, 10, or 30 mg/m^3. Necropsy PE[d].	No significant ($p \leq 0.05$) BALF differences except for dose-dependent presence of mononuclear cells laden with intracytoplasmic crystalline material.	Near NOAEL = 4.4 mg/m^3 for BALF endpoints.
	Male & female rats; age 7 wks; n = 5/sex/group. Nose-only inhalation of basic chromium sulfate dust at 0, 17, 54, or 168 mg/m^3 with exposure duration, Cr(III) equivalent concentrations, and necropsy as above.	≥17 mg/m^3: in male & female BALF, ↓ cells. 168 mg/m^3: in male BALF, ↑ neutrophils and ↓ mononuclear cells.	LOAEL[b] = 17 mg/m^3 for ↓ total BALF cells in males & females.

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; BALF – bronchoalveolar lavage fluid; Cr(III) – trivalent chromium; LOAEL – lowest observable adverse effect level; NOAEL – no observable adverse effect level; PE – post exposure; WB – whole body.

(a) Derived by the original authors unless otherwise noted.

(b) According to review by OEHHA.

(c) It is unclear to OEHHA whether any control animals were included, and whether the reported results are statistically significant.

(d) Amount of time between the last exposure and necropsy not stated by Derelanko *et al*. (1999).

6. Chronic Toxicity

Given OEHHA's chronic RELs are intended to protect the general public over a lifetime of exposure (OEHHA, 2008), chronic and subchronic toxicity of Cr(III) was assessed by OEHHA. Chronic exposures for humans and animal models are considered by OEHHA to occur for at least 12% of the expected lifetime. Average life spans and subchronic/chronic exposure durations are shown in Table 9 below for humans and non-human animal models discussed in this section of the present report.

Table 9. Average life-spans and subchronic exposure durations for humans versus experimental animal models.

Species	Approximate Average Life-span (years)	Subchronic Exposure Duration (weeks)
Human	70	≤364
Rabbit	6	≤31
Rat	2	≤13

Table was modified from Table 7.2.1 by OEHHA (2008b).

6.1 Chronic Toxicity in Humans or Animals

No chronic Cr(III) inhalation studies were identified, and no usable chronic toxicity studies in humans were found by OEHHA. Though there are several occupational studies that have been noted in other government documents (ATSDR, 2012), these studies describe adverse health effects resulting from Cr(VI) or mixed Cr(III)/Cr(VI) exposure. To the best of our knowledge, there were no publicly available peer-reviewed studies of Cr(III) toxicity in chronically exposed humans.

6.2 Sub-chronic Toxicity in Animals

Subchronic Cr(III) studies were performed by Johansson *et al*. (1986a; 1986b) and Derelanko *et al.* (1999) in rabbits and rodents, respectively.

In a series of publications (1986a; 1986b), Johansson *et al*. described the sub-chronic effects of Cr(III) on alveolar Type II cells, lung phospholipid content, lung histopathology, and/or alveolar macrophages. It is unclear to OEHHA whether these publications discuss separate studies. Although the effects of Cr(III) compounds were

compared by Johansson *et al*. to those of other metal compounds, the Cr(III)-related effects are prioritized for discussion herein.

Johansson et al. (1986a)

In this study, male rabbits (2-3 kg) of unstated age and strain (n = 8/group) were exposed in a chamber to a nebulized Cr(III) nitrate nonahydrate [$Cr(NO_3)_3 \times 9\ H_2O$; 98% purity] aerosol of pH = 3, at 0 (filtered air) or 0.6 ± 0.4 mg/m^3 (mean ± SD; 600 ± 400 µg/m^3) for 4-6 weeks (6 hours/day, 5 days/week). The MMAD of the aerosol was approximately 1 µm. Within three days after the last exposure day, animals were sacrificed for collection of lung lobes.

Gross examinations showed that the lungs of Cr-exposed rabbits were normal with no significant weight differences versus controls. However, histopathological assessments of lung tissue sections revealed that 5 of 8 rabbits had increased macrophage accumulations in the intra-alveolar and -bronchiolar regions, Three of 8 rabbits had nodular macrophage granulomas with concomitant but slight lymphocytic influx in the alveolar lumen and interstitium (*i.e*. the area between the alveolar epithelium and the basement membrane of the capillary endothelium). One of 8 rabbits had minor fibrotic nodules ~100 µm in diameter. One control animal was also found to have increased intra-alveolar macrophages and slight but focal interstitial infiltration of lymphocytes and neutrophils.

Ultrastructural findings were mostly unremarkable except for one Cr-exposed rabbit with a nodular accumulation of eosinophils and neutrophils associated with Type II cell proliferation. Volume density of alveolar Type II cells appeared to be higher in Cr-exposed rabbits versus controls, but statistical significance ($p < 0.05$) was not observed. Similar to results in Johansson and Camner (1986), macrophages of Cr-exposed rabbits had numerous lamellated intracellular structures, and large lysosomes containing membranous bodies and distinct black inclusions. Although quantification of lung phospholipids revealed no significant differences between treatment groups, the authors stated that the result was likely due to the short exposure period, and the increased lamellar structures in macrophages may be a first indication of alveolar lipoproteinosis. Pointing to enlarged lysosomes suggestive of disturbed metabolism and unchanging macrophage counts in BALF [macrophage numbers were expected to increase (Johansson *et al*., 1986b).], the authors reiterated that Cr(III) exposure likely affects macrophages directly.

Johansson et al. (1986b)

In this study, the animal model, number of animals per group, and exposures were the same as reported above for Johansson *et al.* (1986a). Exposures occurred in whole-body chambers and rabbits were necropsied within three days of the last exposure for collection and measurement of lung macrophage viability, quantity, metal content, macrophage diameter, oxidative metabolic activity, and phagocytic capability. These biological endpoints were determined by eosin cell staining, a Bürker chamber used for counting cells, scanning electron microscopy with energy-dispersive x-ray spectrometer, a Lanameter microscope generally used for measuring the diameter of fibers, measurement of the reduction of nitroblue tetrazolium (NBT)[24] to formazan in the presence and absence of *Escherichia coli* bacteria, and quantification of the number of fluorescently labeled yeast cells phagocytosed, respectively.

Quantification of total Cr by atomic absorption spectrophotometry and Cr(VI) by a diphenylcarbazide absorption method suggested there was no Cr(VI) present in the Cr(III) aerosol. No significant exposure-related differences in macrophage number, diameter, or viability were observed. Thirty-five percent of rabbits necropsied within three days of the last Cr(III) exposure had macrophages with round dark inclusions, which were shown to contain Cr, in the cytoplasm and/or lysosomes. On average, 90% of macrophages had large lysosomes (>10 μm). Of these cells, 83 ± 10% contained lamellated inclusions – a significant ($p < 0.01$) difference from controls. Decreased cell surface activity, assumed by OEHHA to mean pseudopodia activity, was also observed in macrophages of Cr(III)-exposed rabbits relative to controls, with 29 ± 22% of the observed cells from the former and 6 ± 3% from the latter exhibiting this response. These findings, in combination with enlarged golgi and elongated cell shapes observed more frequently in Cr(III)-exposed rabbits versus controls, were identified by the study authors as important. These can be early responses to increased cellular stress. Further, macrophage metabolic activity was higher in Cr(III)-exposed rabbits versus controls. This was reported as significantly ($p < 0.05$) greater formazan production in NBT tests of the former versus the latter. The pattern was the same irrespective of the presence of *E. coli.* In looking at the Cr(III)- and control-exposed groups individually, the authors noted that the magnitude of the response to *E.coli*, i.e. the difference in formazan production with and without *E. coli*, was smaller ($p < 0.05$) in the Cr(III) group.

It is possible that the Cr(III) exposure merely primed the macrophages, activating them and stimulating pro-inflammatory pathways that resulted in a higher baseline level of

[24] The NBT test is an assay designed to test ROS production by immune cells (e.g. neutrophils and macrophages) that use ROS in their defense against bacteria, etc. In the test, cell-generated ROS cause the reduction of NBT to formazan, which appears as insoluble blue-black deposits in the cells.

ROS. However, when incubated for 30 or 60 minutes with yeast cells, Cr(III)-exposed macrophages phagocytosed significantly ($p < 0.05$) less yeast than control-exposed cells. When considered with the other responses, it is more likely that the Cr(III) exposure caused some level of oxidative stress in the macrophages. All subchronic studies reviewed by Johansson *et al*. are summarized in Table 10 herein.

Derelanko et al. (1999)

Subchronic experiments performed by Derelanko *et al*. (1999) involved 7-week old inbred Fischer 344 rats (n = 15/sex/group) exposed nose-only to 1) water-insoluble Cr_2O_3 at 4.4, 15, or 44 mg/m^3 (4400, 15000, or 44000 µg/m^3); 2) water-soluble basic Cr(III) sulfate at 17, 54, or 168 mg/m^3 (17000, 54000, or 168000 µg/m^3); or 3) air for a total of 65 exposures over 13 weeks (6 hrs/day, 5 days/week). Cr(III) equivalent concentrations for both Cr(III) chemicals were 3, 10, or 30 mg/m^3 (3000, 10000, or 30000 µg/m^3) as calculated by the study authors. After the last exposure, 10 IS (immediately sacrificed) rats/sex/group were necropsied while 5 DS (delayed-sacrifice) rats/sex/group were maintained for a 13-week recovery period during which no Cr(III) exposures occurred.

Monitored biological endpoints included: 1) daily clinical observations and weekly BWs taken prior to necropsy in IS and DS rats; 2) clinical pathology including hematology, clinical biochemistry, and urinalysis parameters in IS rats only; 3) urinary *Beta$_2$*-microglobulin (tumor marker) in 5 rats/sex exposed to air, 44 mg/m^3 Cr_2O_3, or 168 mg/m^3 basic Cr(III) sulfate; and 4) tissue pathology in IS and DS rats. It is unclear to OEHHA whether IS or DS rats were used for outcome 3 above. Sperm parameters including motility, count, and morphology were examined in male IS rats only and are summarized in Section 7 of the present document. Statistical analyses included parametric analyses of variance (ANOVAs), Bartlett's tests for homogeneity, Dunnett's t-tests for pairwise comparisons, and/or Welch t-tests with Bonferroni corrections as well as non-parametric Kruskal-Wallis ANOVA and Mann-Whitney U tests, but it is unclear which tests were used for the different endpoints.

Measured aerosol concentrations for Cr_2O_3 and basic Cr(III) sulfate were the same as target concentrations. MMAD ± geometric standard deviation (GSD) of Cr_2O_3 particles were 1.8 ± 1.93, 1.9 ± 1.84, and 1.9 ± 1.78 µm, at the 4.4, 15, and 44 mg/m^3 concentrations, respectively. Those for basic Cr(III) sulfate were 4.2 ± 2.48, 4.2 ± 2.37, and 4.5 ± 2.5 µm for the 17, 54, or 168 mg/m^3 concentrations respectively. MMADs and GSDs were calculated from 21 samples/test group, and no Cr(VI) was detected (limit of detection = 10 ng/mL). The basic chromium sulfate was reported by Derelanko *et al*. (1999) to "readily [form] acidic solutions (pH ≈ 2.8), presumably with the sulfate group."

Although seven rats died during the exposure period, these deaths were stated by Derelanko *et al*. (1999) to be unrelated to the tested chemicals. Six of the seven died due to their exposure restraint tubes and were replaced. One of the seven died for unknown reasons but exhibited "no significant signs of toxicity" upon necropsy. As a whole, results showed that, similar to findings in their subacute study (discussed in Section 5.3 herein), basic Cr(III) sulfate produced greater toxic responses than Cr_2O_3.

No notable clinical observations or significant ($p \leq 0.05$) changes in BW, hematology, serum biochemistry, or urinalysis parameters were reported in Cr_2O_3-exposed rats relative to controls. However, a slight non-significant downward trend in BW was noted during the recovery period for DS males exposed at 44 mg/m^3 versus control. Of the rats exposed to Cr_2O_3, organ weight changes were only observed in female IS groups relative to controls. At ≥15 mg/m^3, there were increases in the mean absolute and relative thyroid/parathyroid weights of the former. Derelanko *et al*. (1999) stated these changes were small and of unknown biological significance without associated gross or microscopic histopathology, but the relative changes amounted to a 20% increase in thyroid/parathyroid weight.

Relative thyroid weights have been reported to decrease with age in Fischer 344 rats (Marino, 2012); thyroid function and associated hormone levels were not assessed by Derelanko *et al*. (1999). Dietary supplementation of Cr(III) picolinate has been shown to interfere with absorption of ingested levothyroxine, a synthetic thyroid hormone used to treat hypothyroidism (John-Kalarickal *et al.*, 2007; PDR, 2020), but OEHHA found no information regarding Cr(III) exposure and hyperthyroidism. Other Cr_2O_3-related effects were limited to the lungs, with histopathologic inflammation and/or hyperplasia correlating to deposits of Cr and accumulations of Cr-laden macrophages in mediastinal and peribronchial lymphoid tissues, tracheal bifurcations, terminal bronchiole-alveolar duct regions, and lung parenchyma of IS and/or DS groups. These impacts are summarized in Table 11 herein.

For rats exposed to basic Cr(III) sulfate, clinical observations of intermittently labored breathing were reported only in female rats exposed at the 168 mg/m^3 concentration. Analysis of BWs revealed significant ($p \leq 0.05$) differences, as rats inhaling basic Cr(III) sulfate at 54 mg/m^3 (males only) or 168 mg/m^3 (males and females) exhibited lower mean BWs than their control counterparts. The BW decline in exposed males continued through the recovery period even though BW gains and food consumption were similar to those in control rats. Hematological and serum biochemistry parameters were also significantly ($p \leq 0.05$) affected by inhalation of basic Cr(III) sulfate at 54 or 168 mg/m^3 (mid- or high-exposure, respectively). These parameters included increased numbers of neutrophils and decreased numbers of macrophages in BALF of males (168 mg/m^3

group only), increased levels of ALP (measured as a biomarker of liver function) in females (168 mg/m^3 group only), and decreased serum cholesterol in females (≥54 mg/m^3). Though female neutrophil and macrophage counts in BALF exhibited similar trends as their male counterparts, there were no statistically significant changes in these parameters relative to controls.

Significant ($p \leq 0.05$), transient organ weight changes associated with basic Cr(III) sulfate, observed in IS rat groups only, were observed in the spleen, brain, liver, kidney, thyroid/parathyroid and testes (Table 12). However, the changes were generally small with no corresponding microscopic histopathology.

Only pulmonary effects persisted through the recovery period to the post-recovery necropsy. These effects included increased mean absolute and relative (to BW) lung/trachea weights in nearly all (IS and DS) rat groups. Microscopic histopathological findings, corresponding to the increased lung weights included 1) chronic alveolar and interstitial inflammation in IS and DS rat groups; 2) mediastinal (in the chest between the sternum and spinal column) lymph node histiocytosis (excessive tissue macrophages) and lymphoid hyperplasia (increased number of lymphocytes in lymph nodes) in all IS and DS rat groups; and 3) granulomatous inflammation in high-exposure DS rats. Edema was not reported.

6.3 Contribution of pH to the Adverse Effects of Acidic Cr(III) Aerosols

In the experiments by Johansson *et al*. (1986a; 1986b) and Derelanko *et al*. (1999) with $Cr(NO_3)_3 \times 9\ H_2O$ and basic Cr(III) sulfate, respectively, the reported health effects may have resulted in part due to pH of the test materials and not solely due to the Cr(III) concentration.

Both groups acknowledged the potential contribution of aerosol pH to their toxicological findings. Derelanko *et al* (1999) stated the more severe and widespread distribution of lesions observed with basic chromium sulfate versus Cr_2O_3 may have been due to the acidity and water solubility of the former. Johansson *et al*. (1986a) hypothesized the actual probability of pH-driven toxicity in their study was low due to neutralization by ammonia in the cages and airways of rabbits. Citing work by Larson *et al*. (1977) in humans, Johansson *et al*. explained that ammonia can convert inhaled sulfuric acid levels of 0.08-1.5 mg/m^3 in the mouth and 0.04-0.13 mg/m^3 in the nose to ammonium sulfate, a relatively less acidic and less toxic sulfate species (Schlesinger, 1989).

Much work has been done regarding the toxicity of inhaled acidic sulfates (NIEHS, 1989). According to Larson *et al*. (1977), expired ammonia concentrations in humans ranged from 7 – 520 µg/m^3. This range overlaps with those of rabbits measured at 10 –

758 μg/m^3 in fed animals and 4 - 236 μg/m^3 in fasted animals with brushed teeth (Vollmuth and Schlesinger, 1984). However, Vollmuth and Schlesinger (1984) pointed out that in most cases, acid neutralization by respiratory ammonia is incomplete and variable depending upon multiple ambient, particle, and physiological factors. Factors mentioned included relative humidity, acid droplet size and surface area to mass ratio, residence time in the respiratory tract, relative concentrations of the acidic sulfate and ammonia, fasted status of the animal/human breathing the aerosol, and bacterial contributions, such that intra- and inter-individual variation were comparable in magnitude. They also noted that since ammonia concentrations are lower in the nose than the mouth, nose-breathing patterns in humans could result in less neutralization than observed in mouth-breathing animal models like rabbits given similar exposure conditions. Thus, OEHHA cannot discount the contribution of pH to the adverse health effects observed upon exposure to acidic Cr(III) species.

Summaries of all the aforementioned subchronic experiments by Johansson, Derelanko, and their respective colleagues are provided in Tables 10 – 12 below.

Table 10. Summary of subchronic inhalation studies in rabbits.

Reference	Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Johansson *et al*. (1986a)	Male rabbits (2-3 kg; unstated age and strain; n = 8/group). WB exposure to nebulized $Cr(NO_3)_3 \times 9\ H_2O$ at 0 (filtered air) or 0.6 ± 0.4 mg/m^3 (mean ± SD) by inhalation, for 4-6 weeks (6 hours/day, 5 days/week). Necropsy ≤3 days PE.	0.6 mg/m^3: macrophage accumulations (5/8), nodular granulomas w/ lymphocytic influx to alveolar lumen and interstitium (3/8), minor fibrotic nodules (1/8), numerous lamellated intracellular structures and large lysosomes containing black inclusions, non-significant trend toward ↑ volume density of Type II cells.	LOAEL[b] = 0.6 mg/m^3 for inflammatory cell influx
Johansson *et al*. (1986b)	Same as Johansson *et al*. (1986a)	0.6 mg/m^3: enlarged golgi, cellular elongation, ↑ metabolic activity and ↓ phagocytic capacity in macrophage	LOAEL[b] = 0.6 mg/m^3 for physical and functional changes in macrophages

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; Cr(III) – trivalent chromium; $Cr(NO_3)_3$ – chromium (III) nitrate; $Cr(NO_3)_3 \times 9\ H_2O$ – chromium (III) nitrate nonahydrate; LOAEL – lowest observable adverse effect level; NOAEL – no observable adverse effect level; PE – post exposure; WB – whole body.

(a) Derived by the original authors unless otherwise noted.

(b) According to review by OEHHA.

Table 11. Summary of subchronic inhalation studies in rats inhaling Cr_2O_3 (Derelanko et al., 1999)

Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Male & female rats (age 7 wks; n = 5/sex/group). Nose-only inhalation of Cr_2O_3 at 0, 4.4, 15, or 44 mg/m^3 for 13 wks (6 hrs/day, 5 days/wk). Cr(III) equivalent concentrations[b] were 0, 3, 10, or 30 mg/m^3. Necropsy 1 day[c] or 13 wks PE of immediate (IS) or delayed (DS) sacrifice groups, respectively.	IS groups ≥4.4 mg/m^3: In males & females, lymph node hyperplasia and dose-dependent increase of intracytoplasmic crystalline material in macrophages. Dense black pigmented Cr accumulations in tracheal bifurcation, peribronchial lymphoid tissue, mediastinal lymph nodes, and macrophages aggregated in random foci in the alveolar lumen, TB-ADJ, and subpleura. Black Cr corresponded to green lung and mediastinal lymph node discoloration observed upon macroscopic evaluation. 15 mg/m^3: In females, ↑ absolute thyroid/parathyroid weights. ≥15 mg/m^3: In males & females, trace to mild chronic interstitial lung inflammation in alveolar septa surrounding Cr-laden macrophages. In males, this was accompanied by Type II cell hyperplasia associated with black Cr deposits and corresponding to increased lung weights at 44 mg/m^3. In females, ↑ relative[d] thyroid/parathyroid weights.	Near-NOAEL = 4.4 mg/m^3 for "low incidence and severity of the pathological effects." LOAEL[e] = 4.4 mg/m^3 for lymph node hyperplasia

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; Cr(III) – trivalent chromium; Cr_2O_3 – chromium (III) oxide; LOAEL – lowest observable adverse effect level; NOAEL – no observable adverse effect level; PE – post exposure; TB-ADJ – terminal bronchiole-alveolar duct junction.

(a) Derived by the original authors unless otherwise noted.

(b) Calculated by Derelanko *et al*. (1999)

(c) Assumed by OEHHA; not stated.

(d) to body weight

(e) According to review by OEHHA.

Table 11. Summary of subchronic inhalation studies in rats inhaling Cr_2O_3 (Derelanko *et al*., 1999; continued).

Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Male & female rats (age 7 wks; n = 5/sex/group). Nose-only inhalation of Cr_2O_3 at 0, 4.4, 15, or 44 mg/m^3 for 13 wks (6 hrs/day, 5 days/wk). Cr(III) equivalent concentrations[b] were 0, 3, 10, or 30 mg/m^3. Necropsy 1 day[c] or 13 wks PE of immediate (IS) or delayed (DS) sacrifice groups, respectively.	IS groups 44 mg/m^3: In males, ↑ absolute and relative[d] lung/trachea weights. DS groups Mostly minimal severity pathology. ≥4.4 mg/m^3: In males & females, persistent green lung and mediastinal lymph node discoloration, and trace to mild Cr-laden macrophages and black pigment in peribronchial lymphoid tissue. In males, persistent black pigment in mediastinal lymph nodes with > incidence versus IS groups; persistent septal cell hyperplasia and interstitial inflammation of ≥ severity to IS groups. ≥15 mg/m^3: In females, persistent trace to mild septal cell hyperplasia and interstitial inflammation. 44 mg/m^3: mediastinal lymph node enlargement	Near-NOAEL = 4.4 mg/m^3 for "low incidence and severity of the pathological effects." LOAEL[e] = 4.4 mg/m^3 for lymphoid hyperplasia of mediastinal lung lymph node

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; Cr(III) – trivalent chromium; Cr_2O_3 – chromium (III) oxide; LOAEL – lowest observable adverse effect level; NOAEL – no observable adverse effect level; PE – post exposure.

(a) Derived by the original authors unless otherwise noted.

(b) Calculated by Derelanko *et al*. (1999)

(c) Assumed by OEHHA; not stated.

(d) to body weight

(e) According to review by OEHHA.

Table 12. Summary of subchronic inhalation studies in rats inhaling basic chromium sulfate (Derelanko et al., 1999).

Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Male & female rats (age 7 wks; n = 5/sex/group). Nose-only inhalation of basic chromium sulfate dust at 0, 17, 54, or 168 mg/m^3 for 13 wks (6 hrs/day, 5 days/wk). Cr(III) equivalent concentrations[b] were 0, 3, 10, or 30 mg/m^3. Necropsy 1 day[c] or 13 wks PE of immediate (IS) or delayed (DS) sacrifice groups, respectively.	IS groups ≥17 mg/m^3: In males & females, ↓ total BALF cells; ↑ cell debris and lysed cells[d]; ↑ absolute and relative[e] lung/trachea weights; histopathology corresponding to lung weight changes including 1) chronic alveolar inflammation with cellular debris, and thickening of alveoli; 2) chronic, intense, and granulomatous multifocal interstitial lung inflammation associated with foreign material and caused by macrophages, multinucleated giant cells, and Type II cell hyperplasia; and 3) trace to severe infiltration of foamy/granular macrophages in the alveolar lumen correlated with gray discoloration. Granulomatous inflammation in the larynx; histiocytosis of peribronchial lymphoid tissue associated with lymph node enlargement; acute nasal inflammation, and suppurative and mucoid exudate. In males, ↓ BW during exposure and recovery periods; and ↓ absolute spleen weights 1 day PE. In females, ↓ serum cholesterol.	LOAEL[f] = 17 mg/m^3 increased lung weights and pathological findings in the respiratory tract

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; BW – body weight; Cr(III) – trivalent chromium; LOAEL – lowest observable adverse effect level; PE – post exposure.

(a) Derived by the original authors unless otherwise noted.

(b) Calculated by Derelanko *et al*. (1999)

(c) Assumed by OEHHA; not stated.

(d) This endpoint did not appear to OEHHA to have been assessed statistically.

(e) to body weight

(f) According to review by OEHHA.

Table 12. Summary of subchronic inhalation studies in rats inhaling basic chromium sulfate (Derelanko *et al*., 1999; continued).

Animal Model & Exposure	Results Relative to Controls	Point of Departure[a]
Male & female rats (age 7 wks; n = 5/sex/group). Nose-only inhalation of basic chromium sulfate dust at 0, 17, 54, or 168 mg/m^3 for 13 wks (6 hrs/day, 5 days/wk). Cr(III) equivalent concentrations[b] were 0, 3, 10, or 30 mg/m^3. Necropsy 1 day[c] or 13 wks PE of immediate (IS) or delayed (DS) sacrifice groups, respectively.	≥54 mg/m^3: In males, ↓ absolute spleen weights. In females, ↓ serum cholesterol. 168 mg/m^3: In males, ↑ BALF neutrophils and ↓ macrophages; ↓ absolute brain and liver weights and ↑ relative[d] brain, kidney, thyroid/parathyroid, and testes weights with no associated microscopic histopathology. In females, sporadic labored breathing during exposure period; ↑ ALP; ↑ absolute and relative[d] thyroid/parathyroid weights, and ↓ absolute spleen weights with no associated histopathology. <u>IS & DS groups</u> ≥17 mg/m^3: In males & females,↑ relative[d] lung/trachea weights; ≥54 mg/m^3: In males & females, ↑ absolute lung/trachea weights; gray lung discoloration <u>DS groups</u> ≥17 mg/m^3: In males & females, mediastinal lymph node enlargement. ≥54 mg/m^3: In males & females, gray mediastinal discoloration; ↑ absolute lung/trachea weights. In males, tan lung focus/foci in the lungs correlated with presence of macrophages	LOAEL[e] = 17 mg/m^3 for increased lung weights and pathological findings in the respiratory tract

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; BW – body weight; Cr(III) – trivalent chromium; LOAEL – lowest observable adverse effect level; NOAEL – no observable adverse effect level; PE – post exposure.

(a) Derived by the original authors unless otherwise noted.

(b) Calculated by Derelanko *et al*. (1999)

(c) Assumed by OEHHA; not stated.

(d) to body weight

(e) According to review by OEHHA..

7. Reproductive and Developmental Effects

OEHHA was unable to find peer-reviewed publications on the reproductive and developmental effects of inhaled Cr(III) in humans. The 1999 study by Derelanko *et al.* was the only one found for non-human animals. As mentioned previously, *Derelanko et al.* exposed Fischer 344 rats (n = 15/sex/group) to water-insoluble Cr_2O_3 at 4.4, 15, or 44 mg/m^3 (4400, 15000, or 44000 µg/m^3), water-soluble basic Cr(III) sulfate at 17, 54, or 168 mg/m^3 (17000, 54000, or 168000 µg/m^3), or air for a total of 65 exposures over 13 weeks (6 hrs/day, 5 days/week). After the last exposure, 10 rats/sex/group were immediately sacrificed, and necropsied for collection of left caudal epididymides and examination of sperm motility, count, and morphology. Minimal details were provided regarding the sperm evaluation methods and results. Disarticulated sperm counts, sperm concentrations, and sperm morphology were determined visually. A total of 200 sperm were examined from each rat for morphology. Intact sperm were evaluated as "normal" or "abnormal," but these subjective terms were not defined by the authors. Findings indicated no exposure-related effects due to Cr_2O_3 or basic Cr(III) sulfate.

Oral studies in animals given high Cr(III) doses via food or drinking water provided conflicting results. While some reported adverse reproductive outcomes related to sperm quality (Zahid *et al*., 1990) and miscarriage, other chronic exposure studies using excessive Cr(III) doses reported no adverse reproductive/developmental effects upon exposure to various Cr(III) compounds (Shara *et al*. 2007; NTP, 2008). Animal studies involving injection of Cr(III) indicated potential of Cr(III) to cross the placenta, deposit in bone, and produce teratogenic skeletal defects (Danielsson *et al*., 1982; Iijima *et al*., 1983). However, these studies are inappropriate for estimating risks via inhalation or oral routes, which exhibit poor absorption.

Epidemiological and experimental studies in humans indicated Cr(III) may be transferred maternally via breast milk, but there was no clear relationship between Cr(III) concentrations in the milk and oral Cr(III) intake (Casey and Hambidge, 1984; Anderson *et al*., 1983; Mohamedshah *et al*., 1998). Thus, existing literature is insufficient for OEHHA to accurately determine reproductive and developmental risks to humans breathing Cr(III). Studies reviewed by OEHHA are briefly summarized in Tables 13-16 covering human breast milk studies, animal food studies, animal gavage/drinking-water studies, and animal injection studies, respectively. It should be noted that these summaries do include all reproductive/developmental toxicity studies involving oral Cr(III) exposure.

Table 13. Summary of breast milk studies in humans.

Reference	Exposure and Population	Measured Biological Endpoints	Results
Casey and Hambidge (1984)	Normal dietary Cr(III) exposure in 45 lactating American women.	Concentration of Cr(III) in whole liquid breast milk [Cr_M]	Mean [Cr_M] = 0.3 µg/L Range [Cr_M] = 0.06 – 1.56 µg/L Majority with [Cr_M] <0.4 µg/L
Anderson *et al*. (1993)	Normal dietary Cr(III) exposure in 17 lactating women 60 days post partum.	Cr(III) intake (Cr_D), and concentration of Cr(III) in serum [Cr_B], urine [Cr_U], and breast milk [Cr_M] measured over 3 days	Maternal Cr_D = 0.79 ± 0.08 µmol/d Control Cr_D ≈ 0.48 ± 0.002 µmol/d Maternal Cr_B = 3.31 ± 0.75 Control Cr_B = 2.5 ± 0.39 Maternal Cr_U = 7.1 ± 1 Control Cr_U = 4.81 ± 0.76 Average [Cr_M] = 0.18 µg/L Statistical correlation between [Cr_B] and [Cr_U]; r = 84. Cr_D not correlated to [Cr_B], [Cr_U], or [Cr_M].
Mohamedshah *et al*. (1998)	6 lactating women given ^{53}Cr for 3 consecutive days and monitored for up to 90 days	Cr_D, [Cr_B], [Cr_U], and [Cr_M] measured on days 8, 10, 15, 30, 60, and 90	[Cr_M] independent of [Cr_D].

Abbreviations: Cr(III) – trivalent chromium;

Table 14. Summary of Cr(III) in food studies with animals.

Reference	Exposure and Population	Measured Biological Endpoints	Results
Zahid *et al*. (1990)	$Cr_2(SO_4)^3$ powder at 0, 100, 200, or 400 ppm and fed (with chow) to male Balb-C Swiss mice for 35 days	Body, testis, and epididymis weights, sperm counts	Decreased numbers of 1) mature/developing sperm cells and 2) normal seminiferous tubules; increased numbers of resting sperm cells, abnormal sperm cells, degenerated seminiferous tubules; undegenerated tubules without spermatogonia; changes in numbers of sperm cells in different meiotic stages
Shara *et al*. (2007)	Male and female rats given 0 or 25 ppm of niacin-bound Cr(III) complex, or 1000 µg elemental Cr(III) daily in feed for 52 weeks. Sacrifice at 26, 39, or 52 weeks.	BW, physical health, eyesight, food/water intake, hematology and clinical chemistry, organ weights and histopathology, hepatic lipid peroxidation	Decreased body weight gains in males and females at the three time-points; no other statistically significant or notable differences from control.
NTP (2008)	Male and female rats and mice given chromium picolinate in feed at 0, 80, 240, 2000, 10,000 or 50,000 ppm for 14 weeks (3 months). N = 10/sex/species/group	Females: vaginal cell differentials and estrous cycle length in females. Males: sperm count and motility; testis and epididymis weights; gross and histopathological examination;	No adverse effects on reproductive tissues

Abbreviations: BW – body weight; Cr(III) – trivalent chromium;

Table 15. Summary of Cr(III) in gavage and drinking-water studies with animals.

Reference	Exposure and Population	Measured Biological Endpoints	Results
Bataineh (1997)	Adult male rats given chromium chloride in drinking water at 1000 ppm for 12 weeks	Sexual behaviors and territorial same-sex aggression	Decreased mounting, increased post ejaculatory interval, increased male-male, decreased weights for testes, seminal vesicles, and preputial glands
Bataineh *et al*. (2007)	Adult female Sprague-Dawley rats given chromium chloride via intragastric intubation, at 25 mg/kg BW on days 1-3 or 4-6 of pregnancy and sacrificed on gestation day 20	# pregnant rats/group; # implantations; # viable fetuses, ratio of resorptions to total implantations	Decreased pregnancies w/ exposure on days 1-3

Abbreviations: BW – body weight; Cr(III) – trivalent chromium;

Table 16. Summary of Cr(III) in injection studies with animals.

Reference	Exposure and Population	Measured Biological Endpoints	Results
Danielsson *et al.* (1982)	Pregnant C57BL dams intravenously injected with 10 µg $^{51}CrCl_3$/g BW in mid or late gestation and sacrificed 1 hour PE.	Maternal transport of Cr(III) to fetus	Accumulations of ^{51}Cr in placental yolk sac and minimally in fetal skeleton. Embryonic concentrations of ^{51}Cr (III) were 0.4% of that in maternal serum.
Iijima *et al.* (1983)	Pregnant mice intravenously injected with $^{51}CrCl_3$ on gestation day 8 and sacrificed at 4, 8, or 12 hours later	Cr(III) transport and embryonic neural development	Embryos exhibiting pyknotic cells on the neural plate; potential neural tube defects

Abbreviations: BW – body weight; Cr(III) – trivalent chromium;

8. Derivation of Reference Exposure Levels

There are no previously existing RELs for Cr(III) and inorganic Cr(III) compounds.

8.1 Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Acute Reference Exposure Level

Study	Henderson *et al*. (1979)
Study population	Syrian hamsters (n = 4/treatment group/time-point; sex and age not stated)
Exposure method	Nose-only inhalation of unstated carrier solvent (control) or nebulized $^{51}CrCl_3$ aerosol at 0, 2.8, or 77 mg/m^3 (0 , 0.26, or 7.1 ppm, respectively)
Exposure continuity	Once
Exposure duration	30 minutes
Critical effects	Enzyme release consistent with cell membrane damage and tissue injury; increased AP, ALP, and β-glucuronidase activity in lung tissue and/or BALF
LOAEL	77 mg/m^3
NOAEL (No observable adverse effect level)	2.8 mg/m^3 (0.26 ppm)
Benchmark concentration	NA
Time-adjusted exposure	$C^n \times T = K = (2.8\ mg/m^3)^1 \times (0.5\ hr/1\ hr) = 1.4\ mg/m^3$
RDDR	0.35
Human Equivalent Concentration (HEC)	$HEC = RDDR \times K = 0.35 \times 1.4\ mg/m^3 = 0.49\ mg/m^3$
LOAEL uncertainty factor (UF_L)	1
Interspecies uncertainty factors	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factors	
Toxicokinetic (UF_{H-k})	√10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	200
Reference Exposure Level	2.5 µg/m^3 (0.0025 mg/m^3)

8.1.1 Summary of Principal Study for Acute REL

RELs are based on the most sensitive and relevant health effects reported in the medical and toxicological literature. Acute RELs are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (OEHHA, 2008).

The Henderson *et al*. (1979) study that reported the results of a 30-minute, nose-only inhalation exposure in Syrian hamsters was evaluated by OEHHA as the basis of the acute REL for Cr (III) and inorganic Cr (III) compounds.

In the study by Henderson *et al*., hamsters were exposed to nebulized $^{51}CrCl_3 \times 6H_2O$ at 0, 2.8, or 77 mg/m^3 for 30 minutes. The particle MMAD ± GSD was 1.7 ± 1.7 µm. Comparison of lung tissue homogenates and BALF from high-exposure hamsters and controls revealed that in the high-exposure hamsters, there was 1) a sharp 75% increase ($p < 0.05$) in tissue AP activity at 1 day PE with resolution to near-control levels on days 7 and 21 PE; 2) an increase of unstated magnitude in tissue β glucuronidase activity at day 1 PE; 3) a doubling of tissue ALP activity at day 21 PE; and 4) an increase in BALF AP activity at days 1, 7, and 21 PE, with variable levels of BALF ALP activity at days 1 and 21 PE ($p < 0.05$ for all stated endpoints).

8.1.2 Determination of the Point of Departure and Associated Adjustments

Associated histopathology in the high-exposure (77 mg/m^3) animals was characterized by the authors as mild, non-specific irritation with no morphological damage. Given the aforementioned findings, the 2.8 mg/m^3 exposure concentration was determined by OEHHA to be a NOAEL and selected as the point of departure (POD). A time-adjusted exposure concentration (K) was then calculated using a modified Haber's Law equation ($C^n \times T = K$) to account for the <1-hour exposure time. In this equation, the variables, C and T represented the experimental exposure concentration (2.8 mg/m^3; 0.26 ppm) and duration (0.5 hours), respectively. Given the lack of an empirically derived value for the Haber's Law exponent (n) of Cr (III), a default value of 1 was assigned, consistent with OEHHA guidelines (2008a), to extrapolate from <1 hour. Thus,
$C^n \times T = K = (2.8 \text{ mg/m}^3)^1 \times 0.5 = 1.4 \text{ mg/m}^3$.

A human equivalent concentration (HEC) was then obtained by calculating a regional deposited dose ratio (RDDR) and multiplying it by K (HEC = RDDR × K). The RDDR is a ratio of fractional particle deposition in the lungs of animals to that in humans. The Multiple-Path Dosimetry Model, which has replaced the RDDR software previously recommended by the US EPA (1994), does not generate RDDRs or HECs for humans using hamster model data. However, OEHHA was able to calculate a HEC using a

modeled RDDR graph from Jarabek (1995) and GetData Graph Digitizer Software (2013; version 2.26.0.20). The RDDR graph is shown in Figure 5 below.

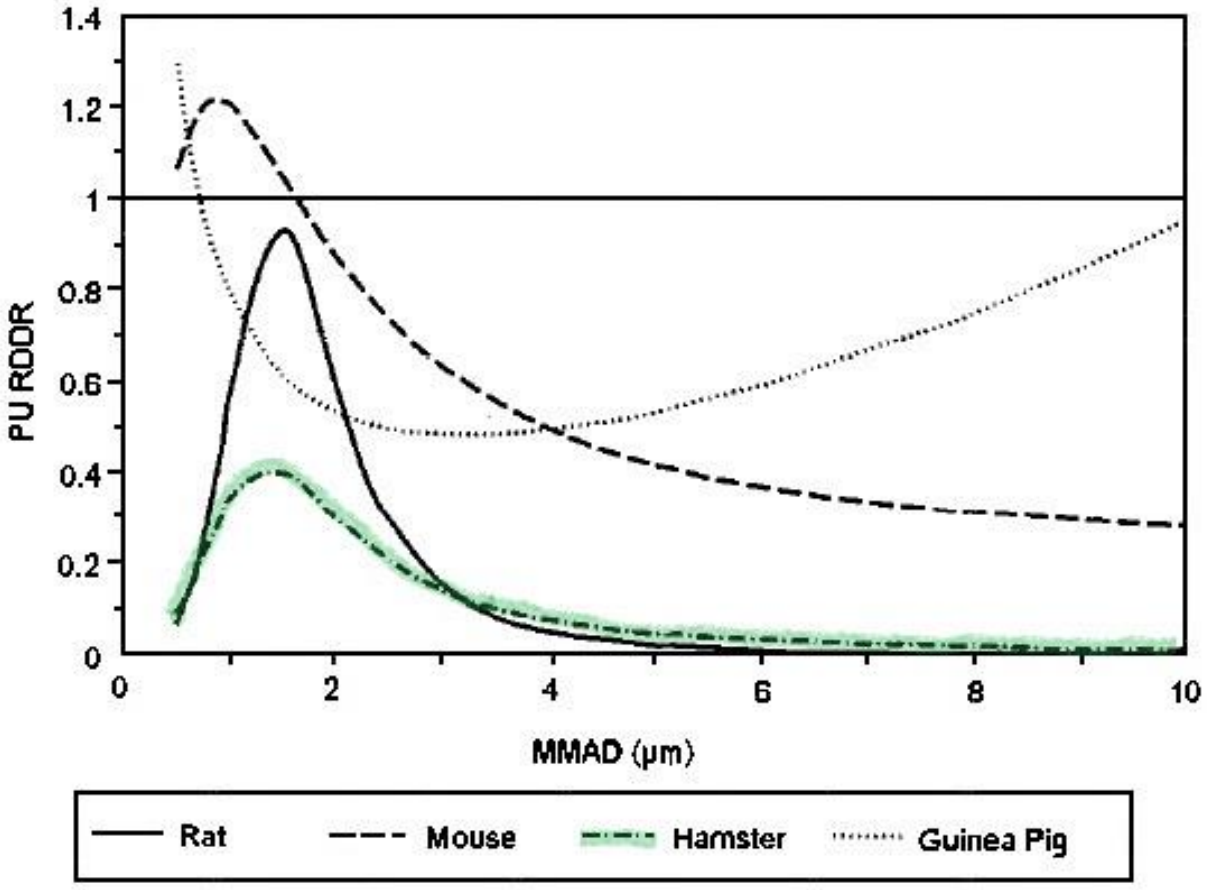

Figure 5. Pulmonary regional deposited dose ratio (PU RDDR) of laboratory animal species to humans. The figure was copied from Jarabek (1995; Figure 3). Ratios are shown for rat, mouse, hamster, and guinea pig models versus humans. The mass median aerodynamic diameter (MMAD) is shown on the x-axis. PU RDDR is shown on the y-axis. The model assumes a geometric standard deviation of 1.73 µm for the particle distribution. Hamster data were highlighted in green by OEHHA. PU RDDR values >1 indicate the human receives a smaller dose than the model animal. Values <1 indicate the human receives a larger dose than the animal model.

The ratios in Figure 5 were calculated by Jarabek (1995) using US EPA (1994) guidance assuming a particle GSD = 1.73 µm. Henderson *et al*. (1979) reported the particle MMAD ± GSD was 1.7 ± 1.7 µm. Thus, OEHHA used Figure 5 with GetData software to determine the hamster-to-human pulmonary RDDR for particles with an MMAD of 1.7 µm. The RDDR obtained by OEHHA using GetData was 0.35 indicating humans would have greater pulmonary deposition than hamsters when breathing

particles with the MMAD and GSD reported by Henderson *et al*. Thus, HEC = RDDR × K = 0.35 × 1.4 mg/m^3 = 0.49 mg/m^3.

A LOAEL uncertainty factor (UF_L) of 1; interspecies toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) uncertainty factors of 2 and √10, respectively; and intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty factors of √10 and 10, respectively were combined for a cumulative UF of 200.

A UF_L of 1 was chosen due to the mild effect, which produced no statistically significant changes in enzyme levels at 2.8 mg/m^3 (Henderson *et al.* (1979), and was consistent with a severity level of 0-1 (OEHHA, 2008). A UF_{A-k} of 2 was used to account for any residual toxicokinetic differences between the non-primate hamster model and humans that were not addressed by the HEC approach. The UF_{A-d} value of √10 was assigned to account for the lack of data on toxicodynamic interspecies differences between the hamster model and humans. A UF_{H-k} of √10 was included to account for variability that may occur due to lower protein binding; hepatic and renal clearance; and metabolic enzyme (e.g. cytochrome P450) activity, abundance, and expression in infants versus adults (Lindeman *et al*., 2000; Louro *et al*., 2000; Lu and Rosenbaum, 2014; Sadler *et al*., 2016); and finally, the UF_{H-d} of 10 was added in consideration of potentially increased sensitivity of children relative to adults during critical developmental windows.

In the study by Henderson *et al.,* lung cell death and tissue damage were observed. Alveolar number, size, and complexity change, exponentially at times, between infancy and adulthood. Insults to the lungs during critical time-frames can produce irrecoverable damage and stunted lung development. Potential for sensitization (Fregert and Rorsman, 1964; Samitz and Shrager, 1966) and exacerbation of asthma (Novey *et al*., 1983; Park *et al*., 1994) were also considered in designation of the UF_{H-d}. Given the cumulative UF of 200, the resulting acute REL for Cr(III) and inorganic Cr(III) compounds was 2.5 µg/m^3, or 0.0025 mg/m^3 (0.0025 mg/m^3 = 0.49 mg/m^3 ÷ 200).

8.2 Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Chronic Reference Exposure Level

Study	Derelanko *et al*. (1999)
Study population	7-week old CDF® (Fischer 344)/Crl BR VAF/Plus® rats (n = 4 - 5/sex/group)
Exposure method	Nose-only inhalation of air or basic Cr(III) sulfate (pH ≈ 2.8) at 17, 54, or 168 mg/m^3
Exposure continuity	6 hrs/day, 5 days/week
Exposure duration	13 weeks
Critical effects	Increased relative lung weights in males due to granulomatous inflammation, Type II cell hyperplasia, and histiocytosis in lymphoid tissue
BMDL	3.83 mg/m^3
Time-adjusted exposure (K)	0.684 mg/m^3 (3.83 mg/m^3 × 6/24 × 5/7)
RDDR	0.3
Human Equivalent Concentration (HEC)	HEC = RDDR × K = 0.3 × 0.684 mg/m^3 = 0.21 mg/m^3
LOAEL uncertainty factor (UF_L)	*1*
Subchronic uncertainty factor (UF_S)	3
Interspecies uncertainty factors	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factors	
Toxicokinetic (UF_{H-k})	√10
Toxicodynamic (UF_{H-d})	10
Cumulative UF	600
Reference Exposure Level	0.342 µg/m^3 (3.42 × 10^{-4} mg/m^3)

8.2.1 Summary of Principal Study for Chronic REL

Chronic RELs are concentrations at or below which adverse health effects are not likely to occur in the general human population exposed continuously over a lifetime. Studies by Johansson *et al*. were unsuitable for REL development because they were missing necessary methodological information, only 4- to 6-week exposure periods, and single-dose level experiments that did not enable determination of a dose-response or NOAEL. However, the study by Derelanko *et al*. (1999) tested water-soluble and water-insoluble Cr(III) compounds at multiple concentrations. Thus, it was used by OEHHA in the chronic and 8-hour REL derivations. The key effect used for development of the chronic REL for inorganic water-soluble Cr(III) compounds was increased lung weights caused by Type II cell hyperplasia and granulomatous inflammation. The key effect for the attempted chronic REL for inorganic water-insoluble Cr(III) compounds was lymphoid hyperplasia. However, a high cumulative uncertainty level prevented development of this REL.

In the study by Derelanko *et al*. (1999), increased lung/trachea weights were noted along with alveolar inflammation, and mediastinal lymph node enlargement with histiocytosis and lymphoid hyperplasia at all tested basic Cr(III) sulfate exposure concentrations (17, 54, or 168 mg/m^3). Given the tested Fischer 344 animal model is known to exhibit increased lung weights with age (Marino, 2012), mean absolute lung weight data were not included in OEHHA's analysis. Though results in the IS groups appeared to be more sensitive indicators of toxicity versus those in the DS groups, data from both time-points were assessed. Data (mean ± SD lung weights) used by OEHHA are shown in Table 17 below.

Table 17. Lung/trachea weights at terminal sacrifice of rats exposed to different concentrations of basic chromium (III) sulfate.

Biological Endpoint	Control; 0 mg/m^3	Low 17 mg/m^3	Mid 54 mg/m^3	High 168 mg/m^3
Relative Weight in Males at 1 day PE (% × 10)	4.42 ± 0.187	5.60 ± 0.271‡	7.15 ± 0.252‡	10.69 ± 0.688‡
Relative Weight in Males at 13 weeks PE (% × 10)	3.89 ± 0.214	4.66 ± 0.373‡	6.37 ± 0.298‡	8.77 ± 0.274‡
Relative Weight in Females at 1 day PE (% × 10)	5.65 ± 0.418	6.99 ± 0.619‡	9.24 ± 1.036‡	12.89 ± 1.134‡
Relative Weight in Females at 13 weeks PE (% × 10)	4.74 ± 0.384	5.75 ± 0.315†	8.02 ± 0.750‡	13.34 ± 0.614‡

Table summarizes results from Derelanko *et al*. (1999), wherein rats were exposed to basic chromium (III) sulfate for 13 weeks and necropsied at 1 day or 13 weeks post exposure. N = 14-15/sex/treatment group at terminal sacrifice and 5/sex/group at the recovery sacrifice. Lung/trachea weights shown above are group means ± standard deviations.
Abbreviations: Cr – chromium; PE – post exposure.
†/‡$p < 0.05$/$p < 0.01$; however, it is unclear to OEHHA whether the reported *p*-value is the result of a parametric analysis of variance (ANOVA) and *post-hoc* Dunnett's t-test for pairwise comparisons, Welch's t-test and *post-hoc* Bonferroni correction; or non-parametric Kruskal-Wallis ANOVA and *post-hoc* Mann-Whitney U-test.

8.2.2 Determination of the Point of Departure and Associated Adjustments

US EPA's (2019) Benchmark Dose Software (BMDS version 3.1.1) was used to determine the benchmark response (BMR) and its 95% lower CI ($BMCL_{1SD}$). The BMR is 1 SD from the control mean. For public health protection, OEHHA used the $BMCL_{1SD}$ as the POD. US EPA (2012) recommends setting the BMR at 1 SD from the control mean when there is no minimum level of change that is generally considered to be biologically significant for a chosen endpoint, and individual data are not available.

BMDS runs were performed using continuous Exponential (M2-M5), Hill, Power, Polynomial (2° and 3°), and Linear restricted and unrestricted models with homo- and hetero-scedastic (same and different variance) assumptions. Five viable models were recommended. These models had the lowest $BMCL_{1SD}$ and AIC (Akaike information

criterion)[25] values when compared to other models from the same data set, and their BMR and/or $BMCL_{1SD}$ values were no more than 1.9 to 5.2 times lower[26] than the lowest non-zero dose from the study by Derelanko *et al*. (1999).

Table 18. Comparison of viable models shown by the United States Environmental Protection Agency's Benchmark Dose Software (BMDS; version 3.1.1) using data from basic Cr(III) sulfate exposures in rats.

Biological Endpoint	Model Type	BMR (mg/m^3)	$BMCL_{SD}$ (mg/m^3)	AIC	*p*-value
Relative Lung/Trachea Weight in Males (13 weeks PE)	Polynomial (2°); Homoscedastic; Unrestricted	4.99	3.83	11.9	0.482
Same as above	Polynomial (2°); Heteroscedastic; Unrestricted	4.98	3.27	13.9	0.482
Relative Lung/Trachea Weight in Females (1 day PE)	Exponential (4); Heteroscedastic; Restricted	5.24	3.62	96.8	0.99
Relative Lung/Trachea Weight in Females (13 weeks PE)	Polynomial (2°); Homoscedastic; Unrestricted	7.53	5.64	36.06	0.78
Same as above	Polynomial (2°); Heteroscedastic; Unrestricted	5.40	3.55	36.13	0.68

Table summarizes results from one BMDS run using lung/trachea weights (mean ± standard deviation) from Derelanko *et al*. (1999), wherein rats were exposed to basic chromium (III) sulfate at Cr (III) equivalent concentrations of 0, 17, 54, or 168 mg/m^3 for 13 weeks and sacrificed 1 day or 13 weeks later. Datasets from the terminal (1 day PE) sacrifice had an n = 9-10/sex/treatment group, and those from the recovery sacrifice (13 weeks PE) had an n = 5/sex/treatment group.
Abbreviation: AIC - Akaike information criterion; BMR – benchmark response; $BMCL_{1SD}$ – 95% lower confidence limit for the BMR; PE – post exposure

[25] AIC values are estimators that allow for qualitative comparison of a group of models using a similar fitting method (continuous, in this case). When multiple usable models are found for the same data set, the model with the lowest AIC would be the presumptive better model (US EPA, 2016).
[26] As the magnitude of the difference between the BMR/$BMCL_{1SD}$ and the lowest non-zero exposure concentration increases, confidence in the modeled BMR/$BMCL_{1SD}$ often decreases reflecting uncertainty about the shape of the exposure-response curve in the low-exposure region. Models with a BMR/$BMCL_{1SD}$ value >10 times lower than the lowest non-zero exposure concentration, for example, are categorized by default as "questionable" versus "viable" in BMDS.

The model chosen by OEHHA for development of the chronic REL was the first one listed in Table 18 above. The BMDS output graph is shown in Figure 6 below, with a modeled curve that fits the data well.

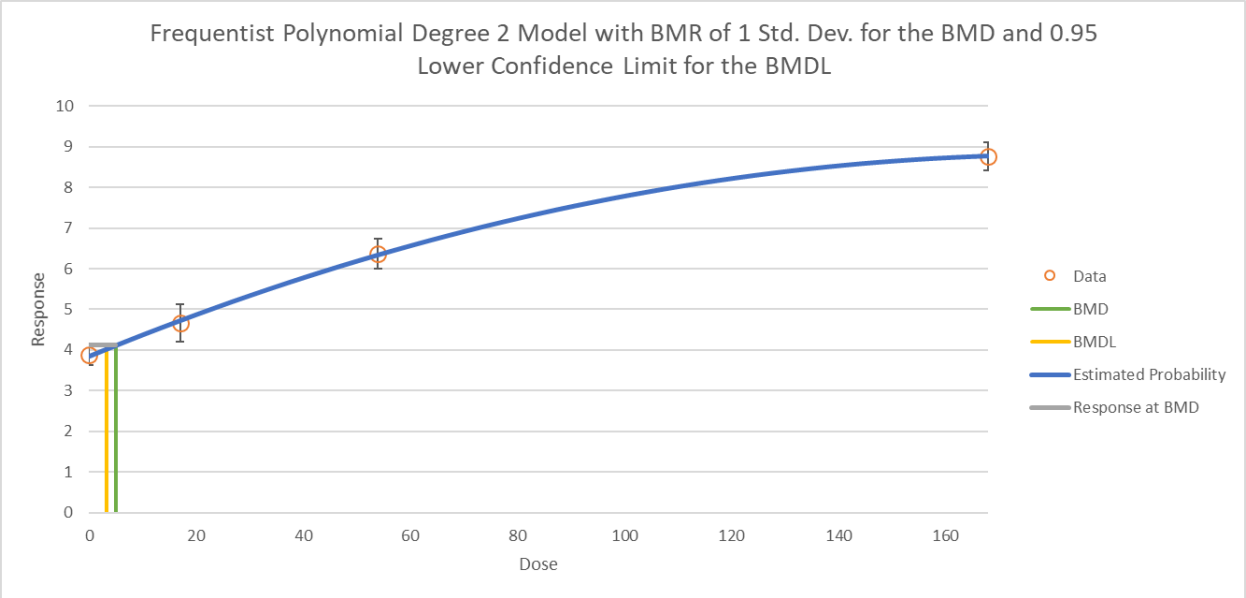

Figure 6. BMDS model POD using male rat lung/trachea weights at 13 weeks post exposure to Cr(III) sulfate. Data were taken from Derelanko *et al.* (1999). The model was generated by the United States Environmental Protection Agency's Benchmark Dose Software (BMDS; version 3.1.1) assuming constant variance among the treatment groups and using a benchmark response (BMR) of one standard deviation from the control mean, and the 95% lower confidence limit of the BMR for the benchmark confidence level ($BMCL_{1SD}$). The BMR and $BMCL_{1SD}$ are shown as BMD and BMDL, respectively, in the figure above.

OEHHA used the $BMCL_{1SD}$ value (3.83 mg/m^3) as the POD, and for the purposes of the chronic REL, calculated a time-adjusted exposure concentration. OEHHA's (2008) default approach for estimating an equivalent inhalation-weighted average concentration (C_{AVG}) from the observed concentration (C_{OBS}) for continuously exposed experimental animals may be summarized by the equation, $C_{AVG} = C_{OBS} \times$ (H hours/24 hours) × (D days/7 days) = K. Using the $BMCL_{1SD}$ and the exposure continuity from the 1999 study by Derelanko *et al.*, the time-adjusted exposure, C_{AVG} = 3.83 mg/m^3 × (6/24) × (5/7) ≈ 0.684 mg/m^3.

Next, an RDDR of 0.3 was calculated (Attachment B). This was used to determine the HEC of 0.21 mg/m^3, which was then adjusted to account for uncertainties. A UF_L of 1 was used since a $BMCL_{1SD}$ was used as the POD. A subchronic uncertainty factor (UF_s)

of 3 was applied to account for a 13-week study duration, approximately 12% of the lifespan of a rat. $UF_{A\text{-}k}$ and $UF_{A\text{-}d}$ of 2 and √10, respectively, were also applied to account for the use of a HEC and limited chemical- and species-specific data in the literature. $UF_{H\text{-}k}$ and $UF_{H\text{-}d}$ of √10 and 10, respectively, were applied to account for human diversity and protect infants and children. There were no data to refute that these youth subpopulations are at higher risk due to differences in toxicokinetics. It is important to account for increased susceptibility of children to adverse respiratory effects like asthma during developmental windows (OEHHA, 2008). In this case, a total UF of 600 was used to adjust the HEC yielding a chronic REL of 0.342 µg/m^3 (0.21 ÷ 600 = 3.42 × 10^{-4} mg/m^3 = 0.0003 mg/m^3).

In attempting to derive a chronic REL for inorganic water-insoluble Cr(III) compounds, OEHHA was limited by a lack of appropriate studies. Though the study by Derelanko *et al*. (1999) included groups of animals exposed to multiple different Cr_2O_3 concentrations, there were no statistically significant continuous or dichotomous dose response data that could be used for a BMDS-based REL derivation. In some cases, such as the increased relative thyroid weights observed in IS females exposed at ≥15 mg/m^3, the organ weight changes could not be correlated to histopathology, or other measured biological parameters that could indicate an exposure-related adverse effect. In other cases, no viable BMDS models were identified. Additionally, because an experimental NOAEL was not established for IS or DS groups, OEHHA was left with a worst-case scenario in which a LOAEL had to be used to derive a NOAEL for REL development. Given a UF_L of 10 and the same aforementioned subchronic, intraspecies, and interspecies UFs used for water-soluble Cr(III), a total UF >3000 was obtained. A total UF >3000 is generally taken to indicate that the study data are insufficient to support derivation of a REL (OEHHA, 2008). This prevented development of a REL for inorganic water-insoluble Cr(III) compounds.

8.3 Chromium (Trivalent) and Inorganic Water-Soluble Trivalent Chromium Compounds Acute REL 8-hour Reference Exposure Level

Study	Derelanko *et al*. (1999)
Study population	7-week old CDF® (Fischer 344)/Crl BR VAF/Plus® rats (n = 4 5/sex/group)
Exposure method	Nose-only inhalation of air or basic Cr(III) sulfate at 17, 54, or 168 mg/m^3
Exposure continuity	6 hrs/day, 5 days/week
Exposure duration	13 weeks
Critical effects	Increased relative lung weights in males due to granulomatous inflammation, Type II cell hyperplasia, and histiocytosis in lymphoid tissue
BMDL	3.83 mg/m^3
Time-adjusted exposure (K)	1.37 mg/m^3 (3.83 mg/m^3 × 6/24 × 5/7 × 20/10)
RDDR	0.3
Human Equivalent Concentration (HEC)	HEC = RDDR × K = 0.3 × 1.37 mg/m^3 = 0.41 mg/m^3
LOAEL uncertainty factor (UF_L)	*1*
Subchronic uncertainty factor (UF_S)	3
Interspecies uncertainty factors	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factors	
Toxicokinetic (UF_{H-k})	√10
Toxicodynamic (UF_{H-d})	10
Cumulative UF	600
Reference Exposure Level	0.683 µg/m^3 (6.83 × 10^{-4} mg/m^3)

8.3.1 Determination of the POD and Associated Adjustments

An eight-hour REL is designed to protect against periodic exposure that could occur as often as daily. Calculations for the 8-hour REL were nearly identical to those for the chronic REL except for the time adjustment. In the 8-hour REL derivation, C_{AVG} is based on the assumption that half of the 20 m^3 of air breathed in any 24-hour period is breathed while active at work. Therefore, the default approach to estimating an equivalent inhalation-weighted average concentration (C_{AVG}) for an eight-hour period of elevated activity (such as at work) from the observed concentration (C_{OBS}) for continuously exposed humans or experimental animals is to use the following equation: $C_{AVG} = C_{OBS} \times$ (H hours/24 hours) × (D days/7 days) × (20 m^3/day total exposure ÷ 10 m^3/day occupational exposure). Using the $BMCL_{1SD}$ and the exposure continuity from the 1999 study by Derelanko *et al.,* the time-adjusted exposure, C_{AVG} = 3.83 mg/m^3 × (6/24) × (5/7) × (20/10) ≈ 1.37 mg/m^3.

9. Evidence for Differential Sensitivity of Children

Under Health and Safety Code Section 39669.5, OEHHA establishes and maintains a list of Toxic Air Contaminants (TACs) that may disproportionately impact infants and children. OEHHA evaluates TACs for addition to this list as we develop RELs for TACs. Cr(III) has been identified by the CARB as a TAC through the listing of chromium and chromium compounds as Hazardous Air Pollutants. Though OEHHA found no studies concerning the effects of Cr(III) exposure in children, it is likely children would experience similar health effects as adults, possibly to greater severity.

Exposure to Cr(III) or Cr(III) compounds is likely to occur via inhalation, oral, or dermal-to-oral routes. Respiratory effects of Cr(III) in children are likely to be more severe than those in adults owing to a faster breathing rate and immature lung development in the former. A faster breathing rate will influence greater particle deposition in the lungs overall, but especially in the upper airways, where affected bronchi/bronchioles can narrow with asthma and make breathing more difficult. To add to this, alveoli in the parenchymal air exchange region of lungs increase in size, number, and complexity into adulthood increasing the surface area for gas exchange with age. Lung volume, airway length, and airway diameter also increase over this time (Stocks and Sonnappa, 2013). Thus, assaults to the developing respiratory system can result in potentially more severe asthmatic episodes than adults and irrecoverable decrements in lung maturation and function. Studies in Section 5 suggest Cr(III) sensitization may occur by Type 1 and Type 4 reactions, both of which produce inflammatory responses that can result in bronchoconstriction and asthma exacerbation in part through the activation of mast cells.

Immature metabolic/elimination processes and antioxidant defenses could also contribute to the greater susceptibility of infants to oxidant challenges like inhaled Cr(III). Examples include lower protein binding; hepatic and renal clearance; and metabolic enzyme activity, abundance, and expression (Lindeman *et al*., 2000; Louro *et al*., 2000; Lu and Rosenbaum, 2014; Sadler *et al*., 2016).

Although the present document does not explore the oral toxicity of Cr(III), ingestion of contaminated food, water, dust, and/or soil represents another major exposure route. Dermal absorption is expected to be low, but exposure via hand-to-mouth activities is possible. Contact with soil containing Cr(III), for example, may cause transfer to the skin and later hand-to-mouth intake. Children have a relatively higher frequency of hand-to-mouth contacts than adults and are thus more likely to have higher Cr(III) exposure via this route. Levels of activity are also greater for children as is contact with the soil and ground surfaces which all increase potential for hand-to-mouth Cr(III) intake. Transmission of Cr(III) from maternal to fetal/infant circulation during pregnancy and/or lactation is also a notable route of exposure for infants and elimination for adult females (Mertz, 1969; Danielsson *et al.*, 1982; Iijima *et al.*, 1983; Casey and Hambidge, 1984; ATSDR, 2012).

In view of 1) the potential of Cr(III) to produce immune sensitization and allergic asthma (Fregert and Rorsman, 1964; Samitz and Shrager, 1966; Novey *et al*., 1983; Park *et al*., 1994); 2) the higher susceptibility of children to these impacts, especially during critical windows of development; and 3) the likelihood of higher exposures in children due to ingestion, OEHHA considers Cr(III) to be an air toxicant that may disproportionately impact children.

10. References

AAAAI (2019). Methacholine Challenge Test. American Academy of Allergy Asthma and Immunology (AAAAI). Updated Jul 11, 2019. Retrieved Jun 15, 2020, from https://www.aaaai.org/conditions-and-treatments/library/asthma-library/methacholine-challenge.

Aitio A, Jarvisalo J, Kiilunen M, Tossavainen A and Vaittinen P (1984). Urinary excretion of chromium as an indicator of exposure to trivalent chromium sulphate in leather tanning. Int Arch Occup Environ Health 54(3): 241-249.

AMBOSS (2019). Hypersensitivity reactions. Updated 2019. Retrieved 2019, from https://www.amboss.com/us/knowledge/Hypersensitivity_reactions.

Anderson RA, Bryden NA, Patterson KY, Veillon C, Andon MB, Moser-Veillon PB (1993). Breast milk chromium and its association with chromium intake, chromium excretion, and serum chromium. Am J Clin Nutr. 57(4) 519-523.

ARA (2015). Multiple-Path Particle Dosimetry Model (MPPD v 3.04). Applied Research Associates, Inc. (ARA). Retrieved 2020, from https://www.ara.com/products/multiple-path-particle-dosimetry-model-mppd-v-304

Arfsten DP, Aylward LL and Karch NJ (1998). Experimental chromium contact sensitivity in animals. In: Immunotoxicology of environmental and occupational metals. Zelicoff J. T. and Thomas P. Taylor & Francis. Bristol, PA: 77-79.

Assembly CS (2005). Assembly floor analysis of AB-721, metal plating facilities: Pollution Prevention Fund. Date of Hearing: April 12, 2005. Accessed: June 2016. http://leginfo.legislature.ca.gov/faces/billAnalysisClient.xhtml?bill_id=200520060AB721

ATSDR. (2011). *Case studies in environmental medicine (CSEM): Chromium toxicity*. https://www.atsdr.cdc.gov/csem/csem.asp?csem=10&po=10; http://www.atsdr.cdc.gov/csem/chromium/docs/chromium.pdf. Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta GA.

ATSDR. (2012). *Toxicological profile for chromium*. Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta GA. https://www.atsdr.cdc.gov/toxprofiles/tp7.pdf

Balamurugan K, Rajaram R, Ramasami T and Narayanan S (2002). Chromium(III)-induced apoptosis of lymphocytes: Death decision by ROS and SRC-family tyrosine kinases. Free Radic Biol Med 33(12): 1622-1640.

Baral A and Engelken R (2005). Modeling, optimization, and comparative analysis of trivalent chromium electrodeposition from aqueous glycine and formic acid baths. J Electrochem Soc 152(7): C504-C512.

Basaran B, Ulaş M, Bitlisli B and Aslan A (2008). Distribution of Cr (III) and Cr (VI) in chrome tanned leather. Indian J Chem Technol 15: 511-514.

Bataineh H, al-Hamood MH, Elbetieha A and Bani Hani I (1997). Effect of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat. Drug Chem Toxicol 20(3): 133-149.

Bataineh HN, Bataineh ZM and Daradka H (2007). Short-term exposure of female rats to industrial metal salts: Effect on implantation and pregnancy. Reprod Med Biol 6(3): 179-183.

Bregnbak D, Johansen JD, Jellesen MS, Zachariae C, Menné T, and Thyssen JP (2015). Chromium allergy and dermatitis: Prevalence and main findings. Contact Derm. 73(5):261-280.

BWH (2001). Iron transport and cellular uptake. Updated Jan 29, 2001. Retrieved May 27, 2019, from http://sickle.bwh.harvard.edu/iron_transport.html.

Capelli A, Lusuardi M, Cerutti CG and Donner CF (1997). Lung alkaline phosphatase as a marker of fibrosis in chronic interstitial disorders. Am J Respir Crit Care Med 155(1): 249-253.

CARB (2008). 2008 CTI [California Toxics Inventory] Summary Table. Updated December 10, 2008. California Air Resources Board (CARB), Sacramento CA. Retrieved May 22, 2019, from https://arb.ca.gov/toxics/cti/cti2008oct2008_v2.xls.

CARB (2010). Draft 2010 CTI [California Toxics Inventory] Summary Table. Updated November 2013. California Air Resources Board (CARB), Sacramento CA. Retrieved May 22, 2019, from https://www.arb.ca.gov/toxics/cti/cti-2010.xlsx.

CARB (2018). Chrome plating operations. Updated November 29, 2018. California Air Resources Board (CARB), Sacramento CA. Retrieved 2020, from https://ww3.arb.ca.gov/toxics/chrome/chrome.htm.

Casey CE and Hambidge KM (1984). Chromium in human milk from american mothers. Br J Nutr 52(1): 73-77.

Cavalleri A and Minoia C (1985). [Serum and erythrocyte chromium distribution and urinary elimination in persons occupationally exposed to chromium(VI) and chromium(III)]. G Ital Med Lav 7(1):35-8.

CCR (1976). California Code of Regulations (CCR), Title 8. Chapter 4. Subchapter 7. General Industry Safety Orders (GISO), Section 5155. https://www.dir.ca.gov/title8/5155table_ac1.html

ChemSrc (2018). Chromium sulfate, basic, solid. Updated Jan 27, 2020. Retrieved Feb 04, 2020, from https://www.chemsrc.com/en/cas/12336-95-7_260360.html.

Chen J, Eraghi Kazzaz A, AlipoorMazandarani N, Hosseinpour Feizi Z and Fatehi P (2018). Production of flocculants, adsorbents, and dispersants from lignin. Molecules 23(4).

Chroneos ZC, Sever-Chroneos Z, Shepherd, VL (2009). Pulmonary surfactant. An immunological perspective. Cell Physiol Biochem. 25(1): 13-26.

Coogan TP, Squibb KS, Motz J, Kinney P and Costa M (1991). Distribution of chromium within cells of the blood. Toxicol Appl Pharmacol 108(1):157-66.

Corbett GE, Finley BL, Paustenbach DJ and Kerger BD (1997). Systemic uptake of chromium in human volunteers following dermal contact with hexavalent chromium (22 mg/l). J Expo Anal Environ Epidemiol 7(2): 179-189.

Corriden R, Insel PA, Chen Y and Junger WG (2008). E-NTPDase1 and alkaline phosphatase control chemotaxis of human neutrophils by generating adenosine from released ATP. FASEB J 22(1_supplement): 1179.1173-1179.

Costa M and Murphy A (2019). Chapter 11 - Overview of Chromium(III) Toxicology. In: The Nutritional Biochemistry of Chromium (III) (Second Edition). Vincent JB. Elsevier. 341-359.

Danielsson BRG, Hassoun E and Dencker L (1982). Embryotoxicity of chromium: Distribution in pregnant mice and effects on embryonic cells *in vitro*. Arch Toxicol 51(3): 233-245.

Danish EPA (2012). *Annex XV Report: Proposal for a Restriction. Chromium (VI) Compounds*. Danish Environmental Protection Agency.

Derelanko MJ, Rinehart WE, Hilaski RJ, Thompson RB and Loser E (1999). Thirteen-week subchronic rat inhalation toxicity study with a recovery phase of trivalent chromium compounds, chromic oxide, and basic chromium sulfate. Toxicol Sci 52(2): 278-288.

DesMarias TL and Costa M (2019). Mechanisms of chromium-induced toxicity. Curr Opin Toxicol 14: 1-7.

Długosz A, Rembacz K, Pruss A, Durlak M and Lembas-Bogaczyk J (2012). Influence of chromium on the natural antioxidant barrier. Pol J Environ Stud 21(2): 331-335.

Ducros V (1992). Chromium metabolism. Biol Trace Elem Res 32(1): 65-77.

Edel J and Sabbioni E (1985). Pathways of Cr (III) and Cr (VI in the rat after intratracheal administration. Hum Toxicol 4(4): 409-416.

Edigaryan AA, Safonov VA, Lubnin EN, Vykhodtseva LN, Chusova GE and Polukarov YM (2002). Properties and preparation of amorphous chromium carbide electroplates. Electrochim Acta 47(17): 2775-2786.

FAO (1996). Management of waste from animal product processing. Retrieved 2020, from http://www.fao.org/3/X6114E/x6114e05.htm.

Feng W (2007). Chapter 6. The transport of chromium(III) in the body. In: The Nutritional Biochemistry of Chromium 121-137. Elsevier BV.

Fregert S and Rorsman H (1964). Allergy to trivalent chromium. Arch Dermatol. 90(1): 4-6.

FTI (2003). Functional trivalent chromium plating process to replace hexavalent chromium plating. Retrieved Jun 01, 2019, from https://nepis.epa.gov/Exe/ZyNET.exe/P1003H8W.txt?ZyActionD=ZyDocument&Client=EPA&Index=2000%20Thru%202005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C00THRU05%5CTXT%5C00000019%5CP1003H8W.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1.

GetData (2013). Getdata graph digitizer. Retrieved 2019, from http://getdata-graph-digitizer.com/download.php.

Gross PR, Katz SA, Samitz MH (1968). Sensitization of guinea pig to chromium salts. J investig Dermatol. 50(5): 424-427.

Hammond CR (2011). Properties of the elements and inorganic compounds. In: Handbook of Chemistry and Physics. A Ready-Reference Book of Chemical and Physical Data, 92nd Edition. Haynes W. M. and Lide D. R. CRC Press. Boca Raton, FL: 4-59.

Henderson RF, Rebar AH, Pickrell JA and Newton GJ (1979). Early damage indicators in the lung. III. Biochemical and cytological response of the lung to inhaled metal salts. Toxicol Appl Pharmacol 50(1): 123-136.

Hesamedini S and Bund A (2017). Formation of Cr(VI) in cobalt containing cr(iii)-based treatment solution. Surface and Coatings Technology 334.

Huang L, Fan ZT, Yu CH, Hopke PK, Lioy PJ, Buckley BT, Lin L and Ma Y (2013). Interconversion of chromium species during air sampling: Effects of O_3, NO_2, SO_2, particle matrices, temperature, and humidity. Environ Sci Technol 47(9): 4408-4415.

Iijima S, Matsumoto N and Lu CC (1983). Transfer of chromic chloride to embryonic mice and changes in the embryonic mouse neuroepithelium. Toxicology 26(3-4): 257-265.

Ikegami M (2006). Surfactant catabolism. Respirology. 11:S24-S27.

IOM (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Institute of Medicine (IOM, US) Panel on Micronutrients. Washington, DC. National Academies Press (US). Chapter 6, Chromium. Available from: https://www.ncbi.nlm.nih.gov/books/NBK222329/

IPCS. (2009). *Inorganic Chromium (III) Compounds*. World Health Organization (WHO). https://apps.who.int/iris/bitstream/handle/10665/44090/9789241530767_eng.pdf?sequence=1&isAllowed=y

Jarabek AM (1995). The application of dosimetry models to identify key processes and parameters for default dose-response assessment approaches. Toxicol Lett. 79(1): 171-184.

Johansson A and Camner P (1986). Adverse effects of metals on the alveolar part of the lung. Scan Electron Microsc(Pt 2): 631-637.

Johansson A, Lundborg M, Hellström P-Å, Camner P, Keyser TR, Kirton SE and Natusch DFS (1980). Effect of iron, cobalt, and chromium dust on rabbit alveolar macrophages: A comparison with the effects of nickel dust. Environ Res 21(1): 165-176.

Johansson A, Robertson B, Curstedt T and Camner P (1986a). Rabbit lung after inhalation of hexa- and trivalent chromium. Environ Res 41(1): 110-119.

Johansson A, Wiernik A, Jarstrand C and Camner P (1986b). Rabbit alveolar macrophages after inhalation of hexa- and trivalent chromium. Environ Res 39(2): 372-385.

John-Kalarickal J, Pearlman G and Carlson HE (2007). New medications which decrease levothyroxine absorption. Thyroid 17(8): 763-765.

Junger WG (2008). Purinergic regulation of neutrophil chemotaxis. Cellular and molecular life sciences. Cell Mol Life Sci 65(16): 2528-2540.

Kiilunen M, Kivisto H, Ala-Laurila P, Tossavainen A and Aitio A (1983). Exceptional pharmacokinetics of trivalent chromium during occupational exposure to chromium lignosulfonate dust. Scand J Work Environ Health 9(3): 265-271.

Kovalszki A and Weller PF (2016). Eosinophilia. Prim care 43(4): 607-617.

Kwon SC KM, Lee JY, Lee SY, Kang DG, Danilov FI, Protsenko VS, Gordiienko VO, Velichenko AB (2012). Trivalent chromium plating solution and plating method using the same. United States. https://patents.google.com/patent/US20120024714A1/en.

Lachapelle JM and Maibach HI (2009). Patch Testing and Prick Testing: A Practical Guide. Second Edition. Official Publication of the ICDRG [International Contact Dermatitis Research Group]. Springer-Verlag. Berlin, Heidelberg, DEU. Retrieved Jun 15, 2020, from https://epdf.pub/patch-testing-and-prick-testing-a-practical-guide-second-edition-official-public.html.

Lantinga H, Nater JP, and Coenraads PJ (1984). Prevalence, incidence and course of eczema on the hands and forearms in a sample of the general population. Contact Derm. 10(3):135-139.

Larson T, Covert D, Frank R and Charlson R (1977). Ammonia in the human airways: Neutralization of inspired acid sulfate aerosols. Science 197(4299): 161-163.

Levina A and Lay PA (2019). Chapter 9 - Redox Chemistry and Biological Activities of Chromium(III) Complexes. In: The nutritional Biochemistry of Chromium (III) (Second Edition). Vincent JB. Elsevier. 281-321.

Levina A, Pham TH and Lay PA (2016). Binding of chromium(III) to transferrin could be involved in detoxification of dietary chromium(III) rather than transport of an essential trace element. Angew Chem Int Ed Engl 55(28): 8104-8107.

Li H, Zhao Y, Li W, Yang J and Wu H (2016). Critical role of neutrophil alkaline phosphatase in the antimicrobial function of neutrophils. Life Sci 157: 152-157.

Lindeman JH, Lentjes EG, van Zoeren-Grobben D, and Berger HM (2000). Postnatal changes in plasma ceruloplasmin and transferrin antioxidant activities in preterm babies. Biol Neonate. 78(2):73-76.

LOBA Chemie (2014). Chromium (III) sulphate basic, extra pure. Updated Aug 06, 2014. Retrieved Jun 03, 2019, from https://www.lobachemie.com/Inorganic-Salts-2819H/CHROMIUM-III-SULPHATE-BASIC-CASNO-39380-78-4.aspx.

Louro MO, Cocho JA, and Tutor JC (2000). Specific oxidase activity of cord serum ceruloplasmin in the newborn. Clin Chem Lab Med. 38(12):1289-1292.

Lu H and Rosenbaum S (2014). Developmental pharmacokinetics in pediatric populations. J Pediatr Pharmacol Ther. 19(4):262-276.

MAK (2015). Manganese and its inorganic compounds [MAK value documentation, 2011]. In: The MAK-Collection for Occupational Health and Safety. Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany: 12: 293-328.

Marino DJ (2012). Age-specific absolute and relative organ weight distributions for Fischer 344 rats. J Toxicol Environ Health, A 75(24): 1484-1516.

MedlinePlus. Urine 24-hour volume. Updated Jun 03, 2019. Retrieved Jun 07, 2019, from https://medlineplus.gov/ency/article/003425.htm.

Mertz W (1969). Chromium occurrence and function in biological systems. Physiol Rev 49(2): 163-239.

MFMER (2019). Allergy skin tests. Updated 2019. Retrieved 2019, from https://www.mayoclinic.org/tests-procedures/allergy-tests/about/pac-20392895.

Mohamedshah FY, Moser-Veillon PB, Yamini S, Douglass LW, Anderson RA, Veillon C (1998). Distribution of a stable isotope of chromium (53Cr) in serum, urine, and breast milk in lactating women. Am J Clin Nutr. 67(6)1250-1255.

Mokgobu MI, Anderson R, Steel HC, Cholo MC, Tintinger GR and Theron AJ (2012). Manganese promotes increased formation of hydrogen peroxide by activated human macrophages and neutrophils *in vitro*. Inhal Toxicol 24(10): 634-644.

Mokgobu MI, Cholo MC, Anderson R, Steel HC, Motheo MP, Hlatshwayo TN, Tintinger GR and Theron AJ (2015). Oxidative induction of pro-inflammatory cytokine formation by human monocyte-derived macrophages following exposure to manganese *in vitro*. J Immunotoxicol 12(1): 98-103.

NCBI (2019a). Chromium (III), CID = 27668. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved Jun 03, 2019, from https://pubchem.ncbi.nlm.nih.gov/compound/Chromium_III_.

NCBI (2019b). Chromic nitrate, CID = 24598. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved Jun 10, 2019, from https://pubchem.ncbi.nlm.nih.gov/compound/Chromic-nitrate.

NCBI (2020a). Chromic oxide, CID = 517277. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved August 26, 2020, from https://pubchem.ncbi.nlm.nih.gov/compound/517277.

NCBI (2019c). Chromium (III) chloride hexahydrate, CID = 104957. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved Jun 11, 2019, from https://pubchem.ncbi.nlm.nih.gov/compound/Chromium_III_-chloride-hexahydrate.

NCBI (2019d). Chromium hydroxide sulfate, CID = 61561. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved Jun 03, 2019, from https://pubchem.ncbi.nlm.nih.gov/compound/61561.

NCBI (2019e). Chromium sulfate, CID = 24930. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved Jun 03, 2019, from https://pubchem.ncbi.nlm.nih.gov/compound/24930.

NCBI (2020b). Chromium chloride, CID = 6452300. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved 2020, from https://pubchem.ncbi.nlm.nih.gov/compound/Chromium-chloride.

NCBI (2019f). Chromium (3^+); hydrogen sulfate, CID = 21414113. PubChem Database. National Center For Biotechnology Information (NCBI), U.S. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services. Retrieved Jun 03, 2019, from https://pubchem.ncbi.nlm.nih.gov/compound/21414113.

Nico PS, Kumfer BM, Kennedy IM and Anastasio C (2009). Redox dynamics of mixed metal (Mn, Cr, and Fe) ultrafine particles. Aerosol Sci Technol 43(1): 60-70.

NIEHS (1989). Environmental Health Perspectives: Symposium on the Health Effects of Acid Aerosols. Research Triangle Park, NC: National Institute of Environmental Health Sciences (NIEHS).

Nielsen GD and Koponen IK (2018). Insulation fiber deposition in the airways of men and rats. A review of experimental and computational studies. Regul Toxicol Pharmacol 94:242-270.

NIH (2018). Chromium. Updated Oct 2018. National Institutes of Health, U.S. Department of Health and Human Services.Retrieved 2019, from https://hazmap.nlm.nih.gov/category-details?id=7&table=copytblagents.

Novey HS, Habib M and Wells ID (1983). Asthma and ige antibodies induced by chromium and nickel salts. J Allergy Clin Immunol 72(4): 407-412.

NTP. (1996a). *Toxicology and Carcinogenesis Studies of Nickel Oxide in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report No. 451*. National Institutes of Health (NIH). National Toxicology Program (NTP).

NTP. (1996b). *Toxicology and Carcinogenesis Studies of Nickel Sulfate Hexahydrate in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP Technical Report No. 454*. National Institutes of Health (NIH). National Toxicology Program (NTP).

NTP. (2008). *NTP Technical Report on the Toxicology And Carcinogenesis Studies Of Chromium Picolinate Monohydrate (CAS No. 27882-76-4) In F344/N Rats and B6C3F1 Mice (Feed Studies)*. National Toxicology Program (NTP). https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr556.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tr556

OEHHA. (2008). *Technical Support Document for the Derivation of Noncancer Reference Exposure Levels.* Office of Environmental Health Hazard Assessment (OEHHA). http://oehha.ca.gov/media/downloads/crnr/noncancertsdsrp2042408.pdf.

O'Flaherty EJ (1991a). Physiologically based models for bone-seeking elements. I. Rat skeletal and bone growth. Toxicol Appl Pharmacol 111(2): 299-312.

O'Flaherty EJ (1991b). Physiologically based models for bone-seeking elements. II. Kinetics of lead disposition in rats. Toxicol Appl Pharmacol 111(2): 313-331.

O'Flaherty EJ (1991c). Physiologically based models for bone-seeking elements. III. Human skeletal and bone growth. Toxicol Appl Pharmacol 111(2): 332-341.

O'Flaherty EJ (1993). Physiologically based models for bone-seeking elements. IV. Kinetics of lead disposition in humans. Toxicol Appl Pharmacol 118(1): 16-29.

O'Flaherty EJ (1995). Physiologically based models for bone-seeking elements. V. Lead absorption and disposition in childhood. Toxicol Appl Pharmacol 131(2): 297-308.

O'Flaherty EJ (1996). A physiologically based model of chromium kinetics in the rat. Toxicol Appl Pharmacol 138(1): 54-64.

O'Flaherty EJ, Kerger BD, Hays SM, Paustenbach DJ (2001). A physiologically based model for the ingestion of chromium (III) and chromium (VI) by humans. Toxicol Sci 60: 196-213.

OHS (2018). The Oregon Encyclopedia: Chromite Mining. Updated March 17, 2018. Retrieved 2019, from https://oregonencyclopedia.org/articles/chromite_mining/#.XPR7Oo97mUl.

Onkelinx C (1977). Compartment analysis of metabolism of chromium(III) in rats of various ages. Am J Physiol 232(5): E478-484.

Park HS, Yu HJ, and Jung KS (1994). Occupational asthma caused by chromium. Clin Exp Allergy. 24(7): 676-681.

PDR (2020). Thyroid-drug summary. Prescribers' Digital Reference (PDR). Retrieved 2020, from https://www.pdr.net/drug-summary/armour-thyroid?druglabelid=2466.

Pinkerton KE, Barry BE, O'Neil JJ, Raub JA, Pratt PC, and Crapo JD. Morphologic changes in the lung during the lifespan of Fischer 344 rats. Am J Anat 164: 155-174.

Proctor DM, Fredrick MM, Scott PK, Paustenbach DJ, and Finley BL (1998). The prevalence of chromium allergy in the United States and its implications for setting soil cleanup: A cost-effectiveness case study. Regul Toxicol Pharmacol. 28(1):27-37.

Protsenko V (2014). Electrodeposition from trivalent chromium baths as an environmentally friendly alternative to electroplating from hazardous hexavalent chromium baths. ChemXpress 4(2): 246-252.

Protsenko V and Danilov F (2014). Chromium electroplating from trivalent chromium baths as an environmentally friendly alternative to hazardous hexavalent chromium baths: Comparative study on advantages and disadvantages. Clean Technol Environ Policy 16: 1201-1206.

Protsenko VS, Kityk AA and Danilov FI (2014). Kinetics and mechanism of chromium electrodeposition from methanesulfonate solutions of Cr(III) salts. Surf Eng Appl Electrochem50(5): 384-389.

Quarles CD, Jr., Marcus RK and Brumaghim JL (2011). Competitive binding of Fe^{3+}, Cr^{3+}, and Ni^{2+} to transferrin. J Biol Inorg Chem 16(6): 913-921.

Rademaker M and Forsyth A (1989). Contact dermatitis in children. Contact Derm. 20(2):104-107.

Randall JA and Gibson RS (1987). Serum and urine chromium as indices of chromium status in tannery workers. Proc Soc Exp Biol Med 185(1): 16-23.

Rudzki E and Rebandel P (1996). Contact dermatitis in children. Contact Derm. 34(1):66-67.

Sadler NC, Nandhikonda P, Webb-Robertson BJ, Ansong C, Anderson LN, Smith JN, Corley, RA, and Wright AT (2016). Hepatic cytochrome P450 activity, abundance, and expression throughout human development. Drug Metab Dispos. 44(7):984-991.

Samitz MH, Katz S, Shrager J (1967). Studies of the diffusion of chromium compounds through skin. J Investig Dermatol. 48(6): 514-520.

Samitz MH and Shrager J (1966). Patch test reactions to hexavalent and trivalent chromium compounds. Arch Dermatol. 94(3): 304-306.

Schlesinger RB (1988). Biological disposition of airborne particles: Basic Principles and Application to Vehicular Emissions. In: Air Pollution, the Automobile, and Public Health. Watson A. Y., Bates R. R. and Kennedy D. National Academies Press (US). Washington, D.C.: 239-298.

Schlesinger RB (1989). Factors affecting the response of lung clearance systems to acid aerosols: Role of exposure concentration, exposure time, and relative acidity. Environ Health Perspect 79: 121-126.

Shara M, Kincaid AE, Limpach AL, Sandstrom R, Barrett L, Norton N, Bramble JD, Yasmin T, Tran J, Chatterjee A, Bagchi M and Bagchi D (2007). Long-term safety evaluation of a novel oxygen-coordinated niacin-bound chromium (III) complex. J Inorg Biochem 101(7): 1059-1069.

Shupack SI (1991). The chemistry of chromium and some resulting analytical problems. Environ Health Perspec 92: 7-11.

Sigma-Aldrich (2017). Chromium (III) sulfate basic, v000727. Updated May 05, 2017. Retrieved 2019, from https://www.sigmaaldrich.com/catalog/product/vetec/v000727?lang=en&region=US.

SKC (1996). Sampling Train - Impingers. SKC, Inc. Covington, GA.

Song YB and Chin DT (2002). Current efficiency and polarization behavior of trivalent chromium electrodeposition process. Electrochim Acta 48(4): 349-356.

Staniek H and Wójciak RW (2018). The combined effects of iron excess in the diet and chromium(III) supplementation on the iron and chromium status in female rats. Biol Trace Elem Res 184(2): 398-408.

Suarez O, Olaya JJ and Rodil S (2012). The effect of operating conditions during plating on the electrochemical behavior and morphology of trivalent solution-derived chromium coatings. Rev Mex Ing Quím 12: 129-141.

Stocks J and Sonnappa S (2013). Early life influences on the development of chronic obstructive pulmonary disease. Ther Adv Respir Dis 7(3): 161-173.

Sun H, Brocato J and Costa M (2015). Oral chromium exposure and toxicity. Curr Environ Health Rep 2(3): 295-303.

Talaei F, Hylkema MN, Bouma HR, Boerema AS, Strijkstra AM, Henning RH and Schmidt M (2011). Reversible remodeling of lung tissue during hibernation in the Syrian hamster. J Exp Biol 214(8): 1276-1282.

Torkmahalleh MA, Lin L, Holsen TM, Rasmussen DH and Hopke PK (2013). Cr speciation changes in the presence of ozone and reactive oxygen species at low relative humidity. Atmos Environ 71: 92-94.

TOXNET (2016). Chromium (III sulfate, CASRN: 10101-53-8. Updated Jan 14, 2016. Retrieved 2019, from https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+10101-53-8.

TURI (2006). *Five Chemicals Alternatives Assessment Study*. Massachusetts Toxics Use Reduction Institute (TURI). University of Massachusetts, Lowell; https://www.turi.org/TURI_Publications/TURI_Methods_Policy_Reports/Five_Chemicals_Alternatives_Assessment_Study._2006

US EPA (1980). Method 13B - Total Fluoride - Specific Ion Electrode. Emissions Measurement Center, Research Triangle Park, NC: United States Environmental Protection Agency (US EPA). Method 13B**:** 6. https://www.epa.gov/emc/method-13b-total-fluoride-specific-ion-electrode.

US EPA (1983). *EPA-600/8-83-014F: Health Assessment Document for Chromium.* United States Environmental Protection Agency (US EPA). Research Triangle Park, NC.

US EPA (1992). *Trivalent and Total Chromium Emissions Evaluation: The True Temper Company, Seneca, South Carolina*. United States Environmental Protection Agency (US EPA). Washington, DC.

US EPA (1994). *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*. United States Environmental Protection Agency (US EPA). Washington, DC.

US EPA (1995). *AP-42: Compilation of Air Emissions Factors, Volume 1: Stationary Point and Area Sources*. 1. 9.15: United States Environmental Protection Agency (US EPA). https://www.epa.gov/air-emissions-factors-and-quantification/ap-42-compilation-air-emissions-factors#5thed.

US EPA (1998). *Toxicological Review of Trivalent Chromium (Cas No. 16065-83-1)*. United States Environmental Protection Agency (US EPA). Washington, DC. http://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0028tr.pdf; https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=28.

US EPA (2010). National Emission Standards for Hazardous Air Pollutant Emissions: Hard and Decorative Chromium Electroplating and Chromium Anodizing Tanks; Group I Polymers and Resins; Marine Tank Vessel Loading Operations; Pharmaceuticals Production; the Printing and Publishing Industry; and Steel Pickling--HCL Process Facilities and Hydrochloric Acid Regeneration Plants. United States Environmental Protection Agency (US EPA). Updated January 29, 2020. Retrieved 2020, from http://www.regulations.gov/#!docketDetail;D=EPA-HQ-OAR-2010-0600;dct=FR%252BPR%252BN%252BO%252BSR.

US EPA (2011). Dermal Exposure Factors. In: Exposure Factors Handbook, EPA/600r-09/052f. Assessment N. C. f. E. United States Environmental Protection Agency (US EPA). Washington, DC: 7i - 7-32.

US EPA (2012). Benchmark Dose Technical Guidance. EPA/100/R-12/001. United States Environmental Protection Agency (US EPA). Retrieved from https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf

US EPA (2014). Method 6800: Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry. Hazardous Waste Test Methods. SW-846. United States Environmental Protection Agency (US EPA). Retrieved Jun 09, 2019, from https://www.epa.gov/hw-sw846/sw-846-test-method-6800-elemental-and-molecular-speciated-isotope-dilution-mass.

US EPA (2016). *Chromium Compounds: Hazard Summary*. United States Environmental Protection Agency (US EPA). Washington, DC. https://www.epa.gov/sites/production/files/2016-09/documents/chromium-compounds.pdf.

US EPA (2019). *Benchmark Dose Software (BMDS) User Manual*. United States Environmental Protection Agency (US EPA). Washington, DC. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=2ahUKEwi5sqeB6qLgAhWI3lQKHf6mAiEQFjABegQICRAC&url=https%3A%2F%2Fwww.epa.gov%2Fsites%2Fproduction%2Ffiles%2F2015-11%2Fdocuments%2Fbmds_manual.pdf&usg=AOvVaw1Xet3EEj-Vxmubc-uRAvY5.

USCB (2018). Quickfacts: California. Retrieved 2019, from https://www.census.gov/quickfacts/CA.

USP (2015). The Pharmacopeia of the United States of America. General Notices and Requirements. Thirty-Eighth Revision and the National Formulary, Thirty-Third Edition. The United States Pharmacopeial (USP) Convention. Retrieved 2020, from https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisions/gn-rb.pdf

van Ketel WG (1984). Low incidence of occupational dermatitis from chromate. Contact Derm. 10(4):249.

Vanoirbeek JA, Hoet PH, Nemery B, Verbeken EK, Haufroid V, Lison D and Dinsdale D (2003). Kinetics of an intratracheally administered chromium catalyst in rats. J Toxicol Environ Health A 66(4): 393-409.

Veien NK, Hattel T, Justesen O, and Nørholm A (1982). Contact dermatitis in children. Contact Derm. 8(6):373-375.

Visek WJ, Whitney IB, Kuhn US, 3rd and Comar CL (1953). Metabolism of Cr^{51} by animals as influenced by chemical state. Proc Soc Exp Biol Med 84(3): 610-615.

Vollmuth TA and Schlesinger RB (1984). Measurement of respiratory tract ammonia in the rabbit and implications to sulfuric acid inhalation studies. Fund Appl Toxicol 4(3, Part 1): 455-464.

Wada O, Manabe S, Yamaguchi N, Ishikawa S and Yanagisawa H (1983). Low-molecular-weight, chromium-binding substance in rat lungs and its possible role in chromium movement. Ind Health 21(1): 35-41.

Weston WL, Weston JA, Kinoshita J, Kloepfer S, Carreon L, Toth S, Bullard D, Harper K, and Martinez S (1986). Prevalence of positive epicutaneous tests among infants, children, and adolescents. Pediatrics 78(6): 1070-1074.

WHO (2000). *Air Quaity Guidelines for Europe, Second Edition.* WHO Regional Publications, European Series, No. 91. Chapter 6: Inorganic Pollutants, Section 6.4: Chromium. https://www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf

WHO (2009). *Concise International Chemical Assessment Document 76: Inorganic Chromium (III) Compounds*. World Health Organization (WHO) Press.

Wiegand HJ, Ottenwälder H and Bolt HM (1984). Disposition of intratracheally administered chromium(III) and chromium(VI) in rabbits. Toxicol Lett 22(2): 273-276.

Wise JTF, Wang L, Xu J, Zhang Z and Shi X (2019). Chapter 10 - Oxidative Stress of Cr(III) and Carcinogenesis. In: The Nutritional Biochemistry of Chromium (III) (Second Edition). Vincent JB. Elsevier. 323-340.

Wright JL, Cosio M and Churg A (2008). Animal models of chronic obstructive pulmonary disease. Am J Phys Lung Cell Mol Physiol 295(1): L1-L15.

Yeh HC and Schum GM (1980). Models of human lung airways and their application to inhaled particle deposition. Bull Math Biol 42: 461-80.

Attachment A – Calculations of $^{51}Cr^{3+}$ Burdens in Hamsters from Henderson *et al.* (1979)

Table A1. Calculations of the Total $^{51}Cr^{3+}$ Body Burden in Syrian Hamsters at Two Hours Post Inhalation of a Nebulized $^{51}CrCl_3$ Aerosol.

Exposure Group	[A] Lung Burden Mean (µg)[a]	[B] Lung Burden SD (µg)[a]	[C] Fractional Lung Deposition Mean[a]	[D] Fractional Lung Deposition SD[a]	[E = A/C] Actual Mean Quotient (µg)[b]	[F=(A+B)/(C-D)] Largest Possible Quotient (µg)[b]	[G=(A-B)/(C+D)] Smallest Possible Quotient (µg)[b]	[H] Largest Difference (µg)[b,c]	[I = E] Total Body Burden Mean (µg)[b]	[J = H] Total Body Burden SD (µg)[b]
Low Dose	0.71	0.19	0.116	0.021	6.12	9.47	3.80	3.35	6.12	3.35
High Dose	20.4	9.7	0.116	0.021	175.86	316.84	78.10	140.98	175.86	140.98

Table uses 2-hour, post-exposure lung burden and fractional lung deposition values reported by Henderson *et al.* (1979) to calculate total body burden. In the Henderson *et al.* (1979) study, hamsters (n = 4/sex/treatment group/time-point) were exposed to $^{51}CrCl_3$ at 0, 2.8 (low dose), or 77 mg/m^3 (high dose) for 30 minutes, and sacrificed two hours, or 1, 7, or 21 days thereafter.

Abbreviation: SD = Standard deviation

(a) Values in this column were taken directly from Henderson *et al.* (1979).

(b) Values in this column were calculated by OEHHA and rounded to two decimal places. Calculations assume a worst-case scenario with the largest SD.

(c) For each exposure group, H = $|E - F|$ or $|E - G|$, whichever is greatest. "||" denotes absolute value.

Table A2. Calculations of the $^{51}Cr^{3+}$ Organ Burden in Syrian Hamsters at Two Hours Post Inhalation of a Nebulized $^{51}CrCl_3$ Aerosol at 2.8 mg/m^3.

Organ	[A] Total Body Burden Mean (µg)[a]	[B] Total Body Burden SD (µg)[a]	[C] Fractional Organ Deposition Mean[b]	[D] Fractional Organ Deposition SD[b]	[E = A*C] Mean Product (µg)[a]	[F=(A+B)*(C+D)] Largest Possible Product (µg)[a]	[G=(A-B)*(C-D)] Smallest Possible Product (µg)[a]	[H] Largest Difference (µg)[a,c]	[I = E] Organ Burden Mean (µg)[a]	[J = H] Organ Burden SD (µg)[a]
Lung	6.12	3.35	0.116	0.021	0.71	1.30	0.26	0.59	0.710	0.588
Pelt	6.12	3.35	0.304	0.05	1.86	3.35	0.70	1.49	1.861	1.493
Kidney	6.12	3.35	0.014	0.014	0.09	0.27	0.00	0.18	0.086	0.180
Liver	6.12	3.35	0.014	0.014	0.09	0.27	0.00	0.18	0.086	0.180
GI Tract	6.12	3.35	0.361	0.082	2.21	4.20	0.77	1.99	2.210	1.987
Depelted Skull	6.12	3.35	0.154	0.038	0.94	1.82	0.32	0.88	0.943	0.876
Carcass Remains	6.12	3.35	0.037	0.011	0.23	0.45	0.07	0.23	0.226	0.228

Table uses 2-hour, post-exposure total body burden calculated by OEHHA (Table A1 above), and fractional organ deposition values reported by Henderson *et al.* (1979) to calculate different organ burdens. In the Henderson *et al.* (1979) study, hamsters (n = 4/sex/treatment group/time-point) were exposed to $^{51}CrCl_3$ at 0, 2.8 (low exposure), or 77 mg/m^3 (high exposure) for 30 minutes, and sacrificed two hours, or 1, 7, or 21 days thereafter. Calculations in the table focus on the low exposure.

Abbreviation: SD = Standard deviation

[a] Values in this column were calculated by OEHHA and rounded to two decimal places (Table A1 above). Calculations assumed a worst-case scenario with the largest SD.

[b] Values in this column were taken directly from Henderson *et al.* (1979).

[c] For each exposure group, H = $|E - F|$ or $|E - G|$, whichever is greatest. "||" denotes absolute value.

Table A3. Calculations of the $^{51}Cr^{3+}$ Organ Burden in Syrian Hamsters at Two Hours Post Inhalation of a Nebulized $^{51}CrCl_3$ Aerosol at 77 mg/m^3.

Organ	(A) Total Body Burden Mean[a]	(B) Total Body Burden SD[a]	(C) Fractional Organ Deposition Mean[b]	(D) Fractional Organ Deposition SD[b]	(E = A*C) Mean Product[b]	[F=(A+B)*(C+D)] Largest Possible Product[b]	[G=(A-B)*(C-D)] Smallest Possible Product[b]	(H) Biggest Difference[b,c]	(I = E) Organ Burden Mean[b]	(J = H) Organ Burden SD[b]
Lung	175.86	140.98	0.116	0.021	20.40	43.41	3.31	23.01	20.400	23.007
Pelt	175.86	140.98	0.304	0.05	53.46	112.16	8.86	58.70	53.462	58.700
Kidney	175.86	140.98	0.014	0.014	2.46	8.87	0.00	6.41	2.462	6.410
Liver	175.86	140.98	0.014	0.014	2.46	8.87	0.00	6.41	2.462	6.410
GI Tract	175.86	140.98	0.361	0.082	63.49	140.36	9.73	76.87	63.486	76.875
Depelted Skull	175.86	140.98	0.154	0.038	27.08	60.83	4.05	33.75	27.083	33.751
Carcass Remains	175.86	140.98	0.037	0.011	6.51	15.21	0.91	8.70	6.507	8.702

Table uses 2-hour, post-exposure total body burden calculated by OEHHA (Table A1 above), and fractional organ deposition values reported by Henderson *et al.* (1979) to calculate different organ burdens. In the Henderson *et al.* (1979) study, hamsters (n = 4/sex/treatment group/time-point) were exposed to $^{51}CrCl_3$ at 0, 2.8 (low exposure), or 77 mg/m^3 (high exposure) for 30 minutes, and sacrificed two hours, or 1, 7, or 21 days thereafter. Calculations in the table focus on the high exposure.

Abbreviation: SD = Standard deviation

[a] Values in this column were calculated by OEHHA and rounded to two decimal places (Table A1 above). Calculations assumed a worst-case scenario with the largest SD.

[b] Values in this column were taken directly from Henderson *et al.* (1979).

[c] For each exposure group, H = $|E - F|$ or $|E - G|$, whichever is greatest. "||" denotes absolute value.

The $^{51}Cr^{3+}$ activity in the liver and kidney (4.0% ± 2.4% of the lung burden) at sacrifice were not included as part of the lung burden since it could be accounted for by absorption from the GI tract. Liver and kidney burden was calculated by OEHHA as 0.03 ± 0.02 µg for the low-dose group, and 0.82 ± 0.88 µg for the high-dose group according to the calculations below.

Low-dose Group Calculations

Lung burden % uncertainty = 0.19 ÷ 0.71 ≈ 0.27 ≈ 27%

Liver & Kidney burden % uncertainty = 0.024 ÷ 0.04 = 0.60 = 60%

Liver & Kidney burden (mass) = (0.71 µg ± 27%) × (0.04 ± 60%) ≈ 0.03 µg ± 87%

≈ 0.03 ± 0.02 µg

High-dose Group Calculations

Lung burden % uncertainty = 9.7 ÷ 20.4 ≈ 0.48 ≈ 48%

Liver & Kidney burden % uncertainty = 0.024 ÷ 0.04 = 0.60 = 60%

Liver & Kidney burden (mass) = (20.4 µg ± 48%) × (0.04 ± 60%) = 0.82 µg ± 108%

= 0.82 ± 0.88 µg

Attachment B – Calculations of the Minute Volume in Rats and the RDDR

I. Rat Minute Volume Calculation

Using natural logs ($\log_e$), OEHHA calculated the respiratory minute volume (MV), the volume of gas inhaled/exhaled from the lungs of rats in one minute. This was done with Equation 1 below, where b_0 and b_1 are species-specific parameters provided by the US EPA (1994; Table 4-6) and OEHHA (2008b; Table F.1.2). The rat BW (0.2 kg) is an estimate of the mean male BW at the end of the study by Derelanko *et al*. (1999; Figure 1).

Equation 1. $\log_e (MV_A) = b_0 + b_1 \log_e (BW)$

$\log_e (MV_A) = -0.578 + 0.821 \times \log_e (0.2)$

$= -1.9$

$MV_A = e^{(-2.45)} = 0.15$ L/min, or 150 mL/min

II. Multiple-Path Particle Dosimetry (MPPD) Modeling and Regional Deposited Dose Ratio (RDDR) Calculations for the Fractional Deposition of Water-Soluble Cr(III) Particles in the Lungs

MPPD software (version 3.04; ARA, 2015) was used to calculate the Cr(III) deposition in the head, tracheobronchial, and pulmonary regions for rats and humans. Clearance was not included. Most input parameters were based upon the Derelanko *et al*. (1999) study on rats exposed to basic Cr(III) sulfate unless otherwise noted. Fractional deposition was used to calculate the RDDR which was then used in the chronic REL derivation.

MPPD Aerosol Properties

Density = 1.57 g/cm^3 @ 25°C (ChemSrc, 2018)

Aspect Ratio = 1 (default for spherical)

MMAD = 4.2 µm

GSD = 2.48 µm

Concentration: 17 mg/m^3 (LOAEL)

MPPD Inhalability Adjustment [fraction] turned on. According to ARA (2015), checking this box multiplies the inhaled concentration by an inhalability factor, an adjustment relevant for particle sizes >3-4 µm for rats and sizes >8 µm for humans. This is because the probability that particles larger than these are inhaled is less than 1.0 and decreases with increasing particle size as a result of inertial effects. The adjustment is incorporated by using expressions for humans and small laboratory animals fitted to empirical data.

MPPD Rat Parameters

Airway Morphometry

Model = Asymmetric Multiple-Path Long-Evans. MPPD software only has modeling options for Long-Evans and Sprague-Dawley rat strains. Though Fischer 344 rats were used in the study by Derelanko *et al*. (1999), previous studies suggest the surface area of the lungs for a Fischer 344 rat more closely resembles that of a Long-Evans versus Sprague-Dawley rat (Pinkerton *et al*., 1982; Nielsen and Koponen, 2018). The multiple-path model incorporates asymmetry in the lung branching structure and calculates deposition at the individual airway level by using detailed, empirically determined information on lung geometry.

FRC (Functional Residual Capacity; the volume of air in the lungs at the end of a normal expiration) = 4 mL (default)

URT Volume (volume of the respiratory tract from the nostril or mouth down to the pharynx) = 0.42 mL (default)

Constant Exposure Scenario

Acceleration of Gravity = 981 cm/s^2 (default)

Body Orientation = Upright

Breathing Frequency = 102 breaths/minute (default)

Tidal Volume = 1.47 mL (Tidal Volume = Minute Volume ÷ Breathing Frequency). Minute Volume = 150 mL/min as calculated in Section I of Attachment B.

Inspiratory Fraction = 0.5 (default)

Pause Fraction = 0 (default)

Breathing Scenario = Nose Only Exposure

MPPD Human Parameters

Airway Morphometry

Model = Yeh/Schum Symmetric. According to ARA (2015), the model uses a symmetric tree for the whole lung as given by Yeh and Schum (1980). Resulting deposition estimates are average values for each generation. The model may be used for regional (Head, TB, Pulmonary) or total deposition results, and its results correspond with results from the other, more realistic lung structures.

FRC = 3300 mL

URT Volume = 50 mL

Constant Exposure Scenario

Acceleration of Gravity = 981 cm/s^2 (default)

Body Orientation = Upright

Breathing Frequency = 12 breaths/minute (default)

Minute Volume = 13,889 mL/min (20 m^3/day; OEHHA, 2008).

Tidal Volume = 1157 mL (Tidal Volume = Minute Volume ÷ Breathing Frequency).

Inspiratory Fraction = 0.5 (default)

Pause Fraction = 0 (default)

Breathing Scenario = Nasal

Table B1. MPPD Output: Fractional Cr(III) deposition in various regions of the head and lungs.

Species	Head	Tracheobronchial	Pulmonary
Rat	0.5114	0.0103	0.0177
Human	0.6856	0.0358	0.1032

Regional Deposited Dose Ratio (RDDR) calculation:

Setting the same exposure concentration for the rats and humans, the RDDR is expressed as a series of three ratios:

RDDR = (SAh / SAa) x (MVa / MVh) x (Fa / Fh)

Where:

SAh = human surface area – pulmonary region (Table F.1.1, OEHHA, 2008)

SAa = animal (rat) surface area – pulmonary region (Table F.1.1, OEHHA, 2008)

VEa = animal (rat) minute volume

VEh = human minute volume

Fa = animal (rat) fractional deposition for a specific lung region

Fh = human fractional deposition for a specific lung region

Calculations for the pulmonary region, which produced the lowest RDDR, are shown below.

RDDR = (540,000 / 3400 cm^2) x (150 / 13,889 ml/min) x (0.0177 / 0.1032) = 0.3