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## Current Understanding of Hexavalent Chromium [Cr(VI)] Neurotoxicity and New Perspectives

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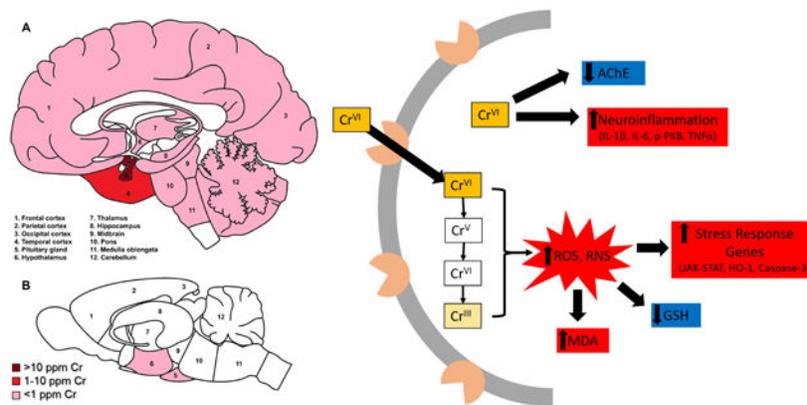
### Abstract

Hexavalent chromium [Cr(VI)] is a global environmental pollutant that increases risk for several types of cancers and is increasingly being recognized as a neurotoxicant. Traditionally, the brain has been viewed as a largely post-mitotic organ due to its specialized composition of neurons, and consequently, clastogenic effects were not considered in neurotoxicology. Today, we understand the brain is composed of at least eight distinct cell types – most of which continue mitotic activity throughout lifespan. We have learned these dividing cells play essential roles in brain and body health. This review focuses on Cr(VI), a potent clastogen and known human carcinogen, as a potentially neurotoxic agent targeting mitotic cells of the brain. Despite its well-established role as a human carcinogen, Cr(VI) neurotoxicity studies have failed to find a significant link to brain cancers. In the few studies that did find a link, Cr(VI) was identified as a risk for gliomas. Instead, in the human brain, Cr(VI) appears to have more subtle deleterious effects that can impair childhood learning and attention development, olfactory function, social memory, and may contribute to motor neuron diseases. Studies of Cr(VI) neurotoxicity with animal and cell culture models have demonstrated elevated markers of oxidative damage and redox stress, with widespread neurodegeneration. One study showed mice exposed to Cr(VI)-laden tannery effluent exhibited longer periods of aggressive behavior toward an “intruder” mouse and took longer to recognize mice previously encountered, recapitulating the social memory deficits observed in humans. Here we conducted a critical review of the available literature on Cr(VI) neurotoxicity and synthesize the collective observations to thoroughly evaluate Cr(VI) neurotoxicity – much remains to be understood and recognized.

### Graphical Abstract

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## Keywords

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## 1. Introduction

Chromium (Cr) is the seventh most abundant element on earth and naturally occurs in two valence states – hexavalent [Cr(VI)] and trivalent [Cr(III)]. While Cr(III) is generally regarded as less toxic and argued to be potentially therapeutic, Cr(VI) is widely recognized as a human carcinogen and an environmental pollutant (Holmes et al., 2008; DesMarias and Costa, 2019; Moreira et al., 2019; Farrokhian et al., 2019; Peng and Yang, 2015; Speer and Wise, Sr., 2018). In the US alone, 900–970 tons of Cr(VI) are released into the air every year, largely due to industrial activities such as burning oil and coal, pigment oxidants, fertilizers, oil well drilling, metal electroplating, and leather tanning (ATSDR, 2012). Occupational exposure poses the most significant risk, and is often encountered in industries that use chromic acid, or are involved in chromite ore production, paint production, or steel welding. Inhalation of Cr(VI) increases incidence and risk of developing lung cancers, while ingestion via drinking water increases risk of liver cancers. As such, Cr(VI) is listed by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen and is listed 17<sup>th</sup> on the Agency for Toxic Substances and Disease Registry's (ATSDR's) Hazards Priority List. Drinking water contaminated with Cr(VI) presents the most wide-spread risk of exposure, though it is currently regulated as total Cr and thus reported drinking water levels for Cr(VI) are sparse. Average Cr(VI) drinking water levels range from 0.2–2 µg Cr(VI)/L in the United States and Canada (US EPA, 2017; Moffat et al., 2018). Sources for Cr(VI) in drinking water may come from erosion of naturally occurring rocks/sediments or anthropogenic industrial pollution (see reviews by Moffat et al., 2018 and McNeill et al., 2012 for further discussion).

Upon entry into the body, Cr(VI) particles dissociate and the Cr(VI) anion crosses cell membranes through ion channels or the entire particulate is phagocytosed. Due to Cr(VI) anion's tetrahedral structure, it mimics sulfate and phosphate ions to passively cross the cell membrane through these ion channels. Interaction of Cr(VI) with arginine in these anion channels is critical for stabilizing the Cr(VI) anion through a network of hydrogen

bonds (Saha et al., 2011). In the cytoplasm, Cr(VI) is rapidly reduced to Cr(III) with ephemeral intermediate valences [Cr (V) and (IV)]. This reduction produces reactive oxygen species (ROS, such as superoxide, hydrogen peroxide, or hydroxyl radicals), which contribute to carcinogenesis. Cr(VI)'s clastogenic effects are well defined, but unknown in the brain (Newbold et al., 1979; Watanabe et al., 1985; Wise, S. et al., 2004). Further, Cr(VI) induces genomic instability, a hallmark of lung cancer, by disrupting DNA damage repair pathways (Wise, S. et al., 2018; Browning et al., 2016; Qin et al., 2014; Wise, S. et al., 2008; Zhitkovich, 2005; Martin et al., 2006; Reynolds et al., 2007).

The brain is a highly specialized organ that demands up to 20% of the body's oxygen supply despite making up less than 2% of body mass. Most often people associate the brain with neurons, and neurotoxicity with how chemicals affect neuronal health. However, there are at least eight distinct cell types in addition to neurons conducting the complexity of the brain, including: astrocytes, microglia, oligodendrocytes, ependymal cells, pericytes, brain vascular endothelial cells, and smooth muscle cells. Collectively, these cell types make up the glia and cerebrovasculature that support the health and maintenance of neurons. Importantly, most of these cells remain mitotic throughout life and hence are potential targets of clastogenic toxicants.

Several brain cells (astrocytes, microglia, pericytes, endothelial cells, and some neurons) work together to form the neurovascular unit (NVU) (Muoio et al., 2014). The NVU detects the needs of the brain parenchyma, triggers appropriate chemotactic responses (e.g. vasodilation, vasoconstriction), and regulates the composition and tightness of the blood-brain barrier (BBB). In a healthy body and healthy brain, the vast majority of xenobiotics are kept out of the brain parenchyma by the BBB (Zheng et al., 2014; Haddad-Tóvulli et al., 2017; Gawdi and Emmady, 2020; Zheng and Ghersi-Egea, 2020). It is unknown if Cr(VI) can cross this barrier, or damage it. Alternatively, Cr(VI) could enter the brain through the olfactory bulb, which is unprotected by the BBB and is directly exposed to ambient air through its nerve fenestrations into the nasal septum (Hanson and Frey, 2008). Additionally, the hypothalamus and pituitary have a weaker BBB due to their roles in hormonal axes (e.g. hypothalamus-pituitary-gonad axis) and thus have regular interactions with blood components (Haddad-Tóvulli et al., 2017). Finally, Cr(VI) could enter the brain through cerebrospinal fluid (CSF). One study investigating metal-on-metal hip arthroplasty observed a small exchange of Co and Cr from blood plasma to CSF (Harrison-Brown et al., 2020). Yet, no data have been reported on Cr(VI) ability to cross the brain-CSF barrier either.

Hexavalent chromium is a widely recognized environmental pollutant with potential for carcinogenic, teratogenic and mutagenic effects. However, understanding the neurological health effects of Cr (VI) exposures are just beginning. Perhaps this is due to a lack of evidence for occupational Cr(VI) exposures linked to major neurodegenerative diseases (e.g. Alzheimer's or Parkinson's diseases). Regardless, we recognize the significant neurological health risks associated with exposure to non-essential heavy metals, and much is yet to be understood about most of these metals (Karri et al., 2016; dos Santos et al., 2016; Su et al., 2017). Our knowledge of brain health and function is growing exponentially with the development of newer imaging tools and more powerful computers to analyze them;

meanwhile our understanding of how our environment affects brain health and function lags behind. Much is yet to be understood about neurotoxicology and how chemicals alter the health and function of the brain (especially non-neuronal cells), and our ability to treat or prevent many diseases will be limited until we do.

The aim of this review is to summarize and discuss the available literature on Cr(VI) neurotoxicity. Our discussion will begin summarizing reports with humans, considering: Cr levels in human brain regions, risk for brain cancer after occupational exposure, Cr metallosis from failed metal-on-metal hip replacements, Cr(VI) and autism spectrum disorder, other neurological disturbances linked to Cr exposures, and the lack of regulations for Cr(VI) neurotoxicity. Then we will discuss reports with animals, considering: dose- and time-dependent Cr deposition in the brain, impacts on animal behavior, oxidative damage, histopathology, and other neurotoxic endpoints. We will discuss comparisons across exposure routes, novel insights considering co-exposures with antioxidants or other metals, and limitations present in the literature for Cr(VI) neurotoxicity. We briefly discuss the observations from cell culture studies. We then discuss studies considering prevention or intervention of Cr(VI) toxicology, current limitations and new insights from Cr(VI) neurotoxicology. We conclude our discussion with future directions for Cr(VI) neurotoxicology research.

## 2. Methods

### 2.1 Search Strategy

International databases (PubMed and Science Direct) and online search engine (Google Scholar) were searched for relevant publications throughout the composition of this review. The first search was executed on 3/11/2020, and the final search was on 1/7/2021. Searches included: 1) ((chromium) and brain) and toxicology, 2) ((chromium) and brain) and disease, 3) (chromium) and brain levels, 4) ((chromium) and neurologic), 5) ((chromium) and neurotoxicity). Additionally, search terms “chromate”, “hexavalent chromium”, “Cr(VI)” were used in place of “chromium”, and “neuro” was used in place of “brain” to avoid missing papers. This search method was enhanced by following referenced literature under consideration in this review, and by considering articles listed under “Cited By” or “Related Articles” links under search results.

### 2.2 Exclusion Criteria

From all results obtained from searches we excluded: 1) articles not relevant to the review topic, 2) articles on pharmacology of trivalent chromium [Cr(III)], 3) articles that may have mentioned Cr as a measurement but did not report Cr data.

## 3. Assessment of Cr Levels in Human Brain

An understanding of the toxic effects of Cr(VI) on the brain starts with determining what normal, non-toxic levels of Cr are present. Here, we discuss brain Cr levels observed in individuals who are presumably not occupationally exposed (occupation was not reported for these studies). The earliest studies from 1975 and 1977 investigated the levels of trace elements including Cr in a few key regions of the brain – the cortex, basal ganglia, and

pituitary (Kanabrocki et al., 1975; Gooddy et al., 1975; Höck et al., 1975; Persigehl et al., 1977). Gooddy et al. (1975) reported a mean brain Cr level of 0.03 µg/g based on 10 patients that died of accidents or cardiac infarction and resided in urban areas. Analysis of brain regions showed mean levels of 0.03 and 0.09 µg/g for the frontal lobe cortex and basal ganglia, respectively, from 2 patients. By analyzing similar brain regions in subjects aged 5 hours to 74 years, Höck et al. (1975) reported cortex and basal ganglia Cr levels ranging from 0.21–1.35 µg/g and 0.43–16.11 µg/g dry weight (0.65–4.09 µg/g and 1.32–28.82 µg/g wet weight), respectively. From their table, there were three individuals aged 64+ and three younger than 25; Cr levels in each of the older individuals was higher than each of the younger individuals, suggesting bioaccumulation across lifespan. Kanabrocki et al. (1975) measured levels of Cr in four of fourteen pituitaries sampled: 0.53, 0.67, 0.33, and 0.83 mg/g dry weight (160.6, 203.03, 100.0 and 251.15 µg/g wet weight) for individuals aged 54, 58, 63, and 72 years, respectively. Finally, Persigehl et al. (1977) measured trace elements in various human organs in three age groups: 0–1, 20–40, and 50–83 and reported the brain Cr levels as 0.8–4.2, <2, and 2.0–9.6 µg/g dry weight (0.24–1.27, <0.61, 0.61–2.91 µg/g wet weight), respectively. While these studies were not able to discriminate the Cr valence state, the >100-fold differences between pituitary and cortex or basal ganglia Cr levels is striking and may reflect the weaker BBB in pituitary or may reflect an unknown essential role for pituitary Cr. These results also may suggest some credence to studies investigating a pharmacological role for Cr(III) in modulating insulin signaling in diabetes and hyperglycemia (Moreira et al., 2019; Farrokhian et al., 2019; Peng and Yang, 2015).

Since these initial studies, several more reports on Cr levels in the brain have been published and are summarized in Table 1 and Figure 1. Markesbery et al. (1984) conducted a thorough examination of trace elements in multiple brain regions across ages from premature births to 85 years old. While the data in the paper was limited, we are able to make some key insights. Levels of Cr were gradually increased with age (except for the 60–79 age group where a decrease was observed), and when data were grouped as adults versus infants, a statistically significant increase in Cr levels was found in adults ( $0.13 \pm 0.01$  vs.  $0.07 \pm 0.02$  µg/g in infants, respectively), suggesting lifespan bioaccumulation. When considering Cr levels regionally in the brain (regions ranged from <0.01 to 0.60 µg/g), considerably more variation across brain regions than other elements was noticed. The highest Cr levels were observed in regions of the cerebellum (0.27 and 0.26 µg/g in the cerebellar hemisphere and vermis, respectively) and lowest levels were observed in the caudate nucleus and putamen (0.05 and 0.08 µg/g, respectively). Unlike the rest of the brain, however, hippocampal Cr levels decreased with age. Rajan et al. (1997) reported a total brain Cr content of  $0.19 \pm 0.03$  mg. Here, the highest Cr level observed was in the temporal cerebrum (0.18 µg/g), an area involved in auditory function and processing information such as speech and words; whereas the lowest level was observed in the hippocampus (0.10 µg/g). Calderón-Guardeñas et al. (2013) reported a unique study design, investigating metal levels in the frontal lobes of individuals living in a heavily-polluted city (Mexico City) vs. those living in low-pollution Mexican cities (Tlaxcala and Veracruz). All brain samples were collected from individuals that died suddenly in accidents and were otherwise healthy. Of the 11 metals measured, Cr was sixth highest reported at  $910 \pm 63$  µg/g and  $527 \pm 85$  µg/g dry weight ( $1210 \pm 84$  µg/g

and  $701 \pm 113 \mu\text{g/g}$  wet weight) for Mexico city residents and low-pollution city residents, respectively.

Only a few studies have reported Cr levels in abnormal brain conditions. Two early studies have reported Cr levels in a total of 17 brain tumors, but neither found a difference between Cr levels in control cortex tissue and either astrocytoma ( $n = 15$ ), oligodendroglioma ( $n = 1$ ), or meningioma ( $n = 1$ ) tissue (Guisin et al., 1991; Civit et al., 2000). However, two recent studies compared levels of trace elements in cerebrospinal fluid (CSF) from Parkinson's disease patients to age-matched controls (Alimonti et al., 2007; Bocca et al., 2004). Both studies observed lower Cr levels in CSF of Parkinson's patients by approximately half that of controls.

In conclusion, Cr accumulates in the human brain and likely bioaccumulates over the course of a lifespan. Two studies observed high levels of Cr in the pituitary and hypothalamus, though it is not clear if this reflects a potential role for Cr in these regions, or if the elevated levels are due to a weaker BBB. Other brain regions observed with high Cr levels were cerebellum and temporal lobe. These areas are not known to be affected by major neurodegenerative diseases, but could have other detrimental impacts on an individual's life if significantly damaged (e.g. impaired speech or locomotion). Further studies are needed to better understand Cr deposition and valence state in the human brain, what contributes to regional differences, and what potential symptoms or health effects might arise from Cr-induced damage.

#### **4. Human Studies Involving Cr(VI) and Neurological or Neurocognitive Effects**

The last few decades have seen a growing number of studies investigating Cr(VI) neurological endpoints in humans, pointing to the onset of paresthesia, depression, gait disturbance, essential tremor, short-term memory loss, difficulty concentrating, impaired olfactory function, and impaired child neuropsychological development (see Table 2 for summary).

##### **4.1 Cr(VI) and Brain Cancer**

Currently, eleven studies have considered links between occupational Cr(VI) exposure and brain cancers; of these, three reported a positive association. Becker et al. (1991) first reported two cases of German welders with at least a 6-month occupational exposure who died of brain tumors, with a risk ratio of 2.7 (95% CI = 0.2–29.3) for Cr(VI). Hara et al. (2010) conducted a cohort study of 1193 Japanese men employed at a Cr plating factory (mean age was 50) between 1970 and 1976, among which 626 workers (52%) had six months or longer working as a Cr plater, while 567 (48%) worked with other metals (i.e. nickel, zinc, precious metal, aluminum). This cohort showed an elevated mortality risk for brain tumors for Cr platers, with a standard mortality ratio (SMR) of 9.14 (95% CI = 1.81–22.09). In sum, three Cr platers had brain tumors compared to only one in the non-Cr plater population. A retrospective case study with Finnish women with brain/CNS cancers and employed during the 1970 census also showed women who were employed as turners

or toolmakers had an estimated standard incidence ratio (SIR) of 11.9 after exposure to 12  $\mu\text{g}/\text{m}^3$  chromates (Wesseling et al., 2002). Importantly, this was the second highest reported SIR in this study; second to aliphatic/alicyclic hydrocarbon solvents at 20 ppm (SIR = 28), but still 10-fold higher than the next risk (1.5  $\text{mg}/\text{m}^3$  of oil mist, SIR 1.80).

Contrary to these reports, eight studies reported a lack of brain cancer risk with occupational Cr(VI) exposure, including two meta-analyses (Cole and Rodu, 2005; Deng et al., 2019). Marsh et al. (2013) evaluated risk of glioblastoma in jet engine manufacturers by a dual design to consider risk from a case-control study and an incidence cohort study from 8 plants in Connecticut, and considered 12 separate components of jet engine fuel, including Cr. They reported 304 cases of workers exposed to a mean of 0.2  $\mu\text{g}/\text{m}^3$  Cr within their case-control study, and 113,439 exposed to 0.24  $\mu\text{g}/\text{m}^3$  Cr within the incidence cohort study. However, their univariate exposure-risk analyses showed no significant risk for Cr in either study design. In a case-control study from the Canadian National Enhanced Cancer Surveillance System, Pan et al. (2005) evaluated the impact of occupational exposures on brain cancer risk. The analysis considered occupational exposure to Cr salts, but found no significance regardless of occupation duration or gender. In a population-based case-control study spanning seven countries (primarily Germany and United Kingdom), Parent et al. (2017) assessed occupational exposure to welding fumes and risk of developing glioma. They reported 178 (9.9%) cases and 359 (7.0%) controls were exposed to Cr, with an odds ratio of 0.9 (95% CI, 0.7–1.1), but no significant effect between any metal and glioma risk. Feingold et al. (1991) investigated the link between childhood cancers (age 0–14) and the parents' job-exposure matrix during the year prior to the child's birth. Their results showed no significant association between either parent's exposure to Cr compounds and child brain cancer, but the authors noted a suggestive dose-response gradient for children with Cr-exposed fathers. Two other studies considered brain cancer risk in jobs known to have significant risk of Cr(VI) exposure. Moulin et al., 1990 considered a cohort of French workers producing ferroalloys and stainless steel, and reported a nonsignificant SMR of 3.36 for CNS tumors (3 CNS tumors observed in 2,269 workers). Danielsen et al. (1996) investigated cancer incidence among Norwegian boiler welders, observed 10 instances of brain cancers in 2,957 welders (SIR = 102; 49–188). Importantly, brain cancers are a common phenotype in metastatic lung cancers (Schuette, 2004). Two studies emphasize this issue, finding 20–30% of non-small cell lung cancer patients developed metastatic brain cancers and both the Kentucky Cancer Registry and the Alberta Cancer Registry reported the highest incidence of brain metastasis from lung cancers (Porta et al., 2011; Villano et al., 2015). Hence it may be the few studies that observed a significant link between Cr(VI) exposure and brain cancer incidence were actually observing metastatic lung cancers, especially considering the vast majority of Cr(VI)-induced lung cancers are squamous cell carcinomas occurring at lung bifurcation sites (Proctor et al., 2014; Speer and Wise, Sr., 2018).

## 4.2 Metal-on-Metal Hip Replacements and Co-Cr Metallosis

The systemic effects of Co-Cr metallosis after failed metal-on-metal (MoM) arthroplasty is thoroughly reviewed elsewhere, and discusses several neurological effects observed including: peripheral neuropathy, sensori-neural hearing loss, visual impairment, and

cognitive decline (Bradberry et al., 2014). Importantly, ~12.5% of MoM replacements require revision surgery within ten years, mostly due to wearing down. With at least 500,000 people receiving MoM hip implants in the U.S. alone (as of 2012), at least 62,000 people will be faced with a MoM hip replacement failure (Rising et al., 2012). This presents a unique exposure route for Co and Cr, where the worn down metal particles can embed into neighboring tissue and slowly leach these metals into the blood. Further, most of these exposures will occur in elderly individuals, who may be differentially susceptible to metal toxicity due to natural aging or co-morbidities. When a patient presents with a variety of symptoms following MoM revision surgery, blood Co and Cr levels are routinely measured, providing a universal reference for comparison across studies. To discuss a few examples; several studies have reported neurological problems in patients exhibiting Cr and cobalt metallosis from 2, 4, or 8 years living with a failed MoM hip replacement (Ikeda et al., 2010; Clark et al., 2014; Green et al. 2017). One report was a case study of a 56-year-old woman exhibiting polyneuropathy two years after a failed MoM hip replacement (Ikeda et al., 2010). This patient exhibited a variety of neurological symptoms including progressive and painful dysesthesia in all extremities, impaired joint sensation, numbness and tingling in distal limbs, auditory disturbance, and was found to have moderate axonal degeneration without inflammation in a sural nerve biopsy. Blood Cr and cobalt levels were reported to be 221 and >400 µg/L, respectively. Two years after replacement surgery the blood metal levels were reduced and the neurological symptoms largely resolved. Green et al. (2017) reported 6 women and 4 men exhibiting neurological deficits following an average of 4 years with a MoM hip replacement failure. Mean blood Cr and cobalt levels were 338 and 669 nmol/L, respectively. Neurological symptoms included depressed mood, short-term memory deficit, location disorientation, difficulty concentrating, and neurocognitive deficit in 7 of the 10 patients. Finally, Clark et al. (2014) reported altered brain structure and function after 8 years of MoM hip replacement failure in 25 men (age 59 ±7) with 1.42 and 1.72 µg/L blood Cr and cobalt, respectively. This study reported benign essential tremor as well as decreased gray matter in the occipital cortex, putamen, and left head of the caudate nucleus. Importantly, Co toxicity can elicit many of the symptoms observed with MoM failed hip replacements, including: cardiomyopathy, hypothyroidism, polycythemia, cognitive dysfunction, neuropathy, and fatigue (Peters et al., 2017). Neurological symptoms observed in patients with failed MoM hip replacements also include essential tremor, peripheral neuropathy, auditory loss, and visual loss not typically observed with Co toxicity alone, and thus may be elicited by Cr neurotoxicity or the interaction of Co and Cr neurotoxicity.

### 4.3 Cr(VI) and Autism Spectrum Disorder

Heavy metal dyshomeostasis is a new perspective thought to contribute to autism spectrum disorders (ASD), as evidenced by multiple studies reporting differential metals levels in ASD children (Jory and McGinnis, 2008; Yasuda et al., 2005; Adams et al., 2006; Yorbik et al., 2010; Skalny et al., 2017a,b). Most of these studies assessed multiple metals in blood, hair, or serum, and frequently reported a significant association between Cr and ASD. A Canadian study of 10 children diagnosed with autism and 15 age-matched control children found elevated Cr levels in red blood cells of autistic children (25.68 ±12.10 vs 21.15 ±13.31 nmol/L, respectively) (Jory and McGinnis, 2008). Six studies analyzed

hair metals in association with ASD; five reported significantly lower Cr levels in ASD children, while one reported elevated levels (Yasuda et al., 2005; Adams et al., 2006; Al-Farsi et al., 2013; Skalny et al., 2017a; Tinkov et al., 2019; Al-Ayahdi, 2005). One study considered subgroups of autism with or without presence of pica, and reported Cr as the most statistically significant of 39 toxic metals assessed and concluded Cr was the most likely factor in etiology of autism with pica (Adams et al., 2006). Another considered ASD subgroups with or without catatonia; they observed elevated hair and serum Cr levels in ASD with catatonia (Tinkov et al., 2019). Their results observed the most significant associations between metal level and ASD with catatonia for Cu, Cr, and V. A Spanish study considered childhood Cr exposure (hair and urine Cr levels) and neuropsychological development for 393 children (171 males, 222 females, mean age 7.8) living in Cr-polluted areas of southern Spain (Caparros-Gonzalez et al., 2019). They concluded even low Cr levels can affect child attention development, and observed high Cr levels linked to poorer performance on neuropsychological tests. Similarly, an earlier study investigated urine levels of heavy metals including Cr, cadmium, and lead in children with autism (30 cases) and healthy children (20 controls). Urine cadmium and lead levels were significantly lower, but urine Cr levels were significantly higher in children with autism than healthy children, suggesting the potential risk of Cr exposure for the children to develop autism (Yorbik et al., 2010). In fact, a population-based case-control study from Pennsylvania identified an association between autism spectrum disorders and *in utero* exposure to elevated ambient Cr(VI) levels, as measured by the National Air Toxics Assessment (Talbot et al., 2015). In sum, there is provocative evidence that Cr plays a role in the etiology of ASD and further investigation is warranted.

#### 4.4 Cr(VI) and Other Neurological Endpoints

The neurotoxicity of Cr(VI) to humans has been considered in a handful of other contexts. Two studies have shown chromate workers in Japan and Korea exhibited olfactory dysfunction correlated to employment duration (Watanabe and Fukuchi, 1981; Kitamura et al., 2003). Importantly, the two studies observed the olfactory impairment was unrelated to septal perforation. Sánchez-Díaz et al. (2018) conducted a geographical analysis of heavy metal pollution in rivers of Spain and 9,434 cases of motor neuron disease deaths. They reported increased risk for motor neuron disease with seven heavy metals; of these metals, Cr was reported to have a 15.7% increased risk and linked to 2,068 deaths (21.9% of total deaths). A recent meta-analysis demonstrated a link between higher blood Cr in patients with acute or newly diagnosed schizophrenia, but was not linked to patients with chronic or previously treated schizophrenia (Saghazadeh et al., 2019). Their results suggest increased Cr levels during early stages of schizophrenia development may cause or aggravate abnormalities of serotonin, contributing to disease burden. One study considered airborne metal emissions and adjudication of youth for felonies, reporting a statistically significant association for five metals considered, with the exception of Cr (Haynes et al., 2011).

#### 4.5 Regulations Lacking Considerations for Cr(VI) Neurotoxicity

Neurotoxic effects are not considered in any safety regulations for Cr(VI) exposure. In fact, the “Toxicological Profile for Chromium” from the Agency for Toxic Substances

and Disease Registry (ATSDR), which is considered the gold standard for toxicological reference, does not consider most of the literature discussed here and lacks much needed information on Cr(VI) neurotoxicology. Safety levels for inhalation exposures are determined with data pertaining to pulmonary health effects from epidemiology or animal studies. However, the results presented by Hegazy et al. (2021) suggest real-life exposures to Cr(VI) pose a significant neurotoxic risk. Here, the authors used intra-nasal instillation of potassium dichromate at concentrations that mimicked what an electroplater might be exposed to (2.4 mg Cr(VI)/kg/d), exposing rats to 0.125, 0.25 or 0.5 mg/kg/d for 5 consecutive days and evaluated neurotoxic endpoints after 2 weeks, 1 month, or 2 months of this exposure regiment. Their results demonstrated behavioral and neuropathological effects that are discussed later. EPA standards for drinking water defines a chronic oral reference dose of 0.003 mg Cr(VI)/kg/d, based on a study with rats exposed to 2.5 mg Cr(VI)/kg/d in drinking water for 1 year (ATSDR, 2012; MacKenzie et al., 1958). This study did not consider behavior or neurological effects, and there are currently no studies focused on Cr(VI) neurotoxicity at or below 2.5 mg/kg/d for comparison. Finally, two recent review articles reporting on Cr(VI) regulations and health effects failed to mention neurotoxicology as a potential health effect after Cr(VI) exposure (Vaiopoulou and Gikas., 2020; Alvarez et al., 2021). Hence, there is much needed consideration for neurotoxicology in our national and international discussion about Cr(VI) toxicology.

#### 4.6 Summary of Cr(VI) Neurotoxicity Observed in Humans

In sum, these papers demonstrate the need for deeper understanding of Cr(VI) impacts on brain health, development, and neurobehavior (Table 2). Given Cr(VI)'s known roles in carcinogenesis, it is not surprising brain cancer has been considered most often as an endpoint in these human studies. While most of the evidence suggests a lack of association with cancers, some indeed raise the possibility that Cr(VI) may contribute to carcinogenesis in the brain by mechanisms yet to be explored. Two separate groups reported loss of olfaction in chromate workers. This may be an early warning sign for other neurological problems, as hyposmia is a prodromal symptom of Alzheimer's disease and Parkinson's disease. Results from the failed MoM hip replacements were unique in that the Cr(VI) exposure was internal and was in elderly individuals. We do not yet understand the interactions between aging and metals toxicology, but this collection of papers may provide us with a unique glimpse of what to expect for Cr(VI): social memory loss, spatial disorientation, depression, polyneuropathy, and loss of regional brain mass. While these neurological symptoms were recognizable in a clinical setting following surgery, such symptoms could be easily missed in someone environmentally or occupationally exposed to Cr(VI). The handful of other neurological conditions linked to Cr(VI) exposures further reflect our poor understanding of its neurotoxicity; autism spectrum disorder, schizophrenia and motor neuron disease are all distinct neurological conditions with different etiologies.

### 5. Experimental Studies and Potential Mechanisms for Cr(VI) Neurotoxicity

#### 5.1 Animal Models Used for Studying Cr(VI) Neurotoxicity

The neurotoxicity of Cr(VI) has been tested in nine distinct species to date (rat, mouse, rabbit, guinea pig, chicken, zebrafish, rockfish, Japanese quail, and *C. elegans*), primarily

with sodium chromate ( $\text{Na}_2\text{CrO}_4$ ) or potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) administered intraperitoneally or in drinking water. Toxic endpoints are often inconsistent across studies, making comparisons between most studies difficult. The most common endpoints include brain Cr accumulation, oxidative damage (most often assessed by malondialdehyde), and effects on acetylcholinesterase. A few studies considered other endpoints including: behavioral changes, histopathology, neuroinflammation, and brain element homeostasis. The experimental details for animal models reported for Cr(VI) neurotoxicity are summarized in Table 3.

**5.1.1 Cr brain levels in animal models**—Only one study has conducted a thorough assessment for regional distribution of normal brain Cr levels in an animal model; Wasim et al. (2014) reported Cr levels in cerebellum, cerebrum, medulla oblongata, meninges, midbrain, pons, and thalamus from five healthy goats. They reported a whole brain average Cr level of  $0.54 \mu\text{g/g}$  and regional levels ranged from  $0.10$  to  $1.20 \mu\text{g/g}$ , with the highest level observed in the meninges and the lowest in the pons. In addition, Zhu et al. (2018) reported brain Cr levels in chickens from a control group in their investigation of Se-Cr interactions. Groups of five chickens were euthanized after 14, 28, and 42 days in the study exhibited brain Cr levels of  $0.0015 \pm 0.0004$ ,  $0.0026 \pm 0.0003$ , and  $0.0036 \pm 0.0004 \mu\text{g/g}$ , respectively. Intriguingly, their observations suggest Cr bioaccumulation in the chicken brain with age, similar to what was observed in humans.

Eight Cr(VI) neurotoxicity studies have considered brain Cr accumulation in their reports (*Nota Bene*: two studies used a trivalent nanoparticle form,  $\text{Cr}[\text{NO}_3]_3$ , but were included in this review because the focus was on neurotoxicity and not neuropharmacology). These studies included doses ranging from  $2$ – $73.05 \text{ mg/kg}$  with Cr administered either via intraperitoneal (i.p.) injection or drinking water and resulted in brain Cr levels ranging from  $0.06 \mu\text{g/g}$  in the hypothalamus to  $5.4 \mu\text{g/g}$  in whole brain. Generally, i.p. injection of Cr(VI) resulted in higher accumulation in the brain than exposure through drinking water. One study compared i.p. injection to intra-nasal administration, observing similar proportions of Cr deposition relative to administered dose (Salama et al., 2016). Two studies considered multiple exposure times in rabbits and mice and reported similar levels regardless of time, though much is yet to be learned about Cr deposition in the brain over time (Mathur et al., 1977; Ueno et al., 2001). Two studies from the same group considered regional differences of Cr accumulation in the brains of Wistar rats after administering 100 or 500 ppm Cr(VI) in drinking water (Quinteros et al., 2007; Nudler et al., 2009). These reported Cr levels in the pituitary and hypothalamus in comparison to liver levels; liver levels were about 100x higher than brain levels. Comparing the two studies, they observed a 2.3x higher Cr level in both pituitary and hypothalamus after 500 ppm vs 100 ppm Cr(VI) in drinking water. However, these reports did not include cortex or brain homogenate Cr levels, so comparison to other studies is currently limited. Altogether, these studies demonstrate Cr(VI) can readily enter the brain and accumulate with time and prolonged exposure, but there is a severe lack of regional analysis to determine where Cr deposits in the brains of laboratory animals.

**5.1.2 Cr(VI) and neurobehavior**—Behavior is an essential aspect of neurotoxicology analyses in animal studies, as it is the most informative way to link neurotoxicology

health effects across species. Unfortunately, the Cr(VI) literature is brief regarding behavior analyses. Hegazy et al. (2021) used an intra-nasal model to expose rats to 0.125, 0.25, or 0.5 mg Cr/kg/d for five days each week, and assessed neurobehavior impacts after two weeks, one month, or two months of this exposure regiment. They reported dose- and time-dependent effects for: 1) significantly decreased locomotor activity in an activity cage, 2) significantly impaired learning and memory with an object recognition test, and 3) impaired motor coordination. Singh and Chowdhuri (2017) reported dose-dependent decreased climbing ability and jumping activity of fruit flies after 48 h Cr(VI) in their food, also supporting an effect of Cr(VI) on locomotor function and coordination. Heffern et al. (2018) observed no olfactory deficit in zebrafish after 24 hours exposure to potassium dichromate, even up to 2,700 µg/L. After a single intranasal doses of Cr(VI) of 0.5, 1.0 or 2.0 mg/kg, rats exhibited dose-dependent decreased motor activity by 42%, 59%, and 77%, respectively (Salama et al., 2016). One possible cause of this behavior the authors did not consider could be cerebellar toxicity, which was one of the highest Cr-laden regions in human brains; if the rats were uncoordinated they would likely move less. Estrela et al. (2017) observed short-term social memory deficits in female mice exposed to tannery effluent. Here, the authors used tannery effluent provided by an industry in Goiás State, Brazil and reported an effluent Cr level of 859 mg/L (at least 150x higher than other metals). This unique model exposed mice to 500 mL tannery waste for 2 hours per day for 20 consecutive days. In order to test social recognition, an “intruder” mouse was introduced to a resident mouse’s cage for 3 minutes, removed for 15 minutes, then re-introduced for 3 minutes again, with all encounters being recorded. A buried food test was also employed to test olfactory function. The tannery effluent-exposed mice exhibited no difference in the buried food test, but spent less time exploring the anogenital region and longer time displaying aggressive behavior toward an “intruder” mouse on the second encounter. Thus, the olfactory function was intact but their ability to recognize mice they recently encountered was impaired, and the authors concluded that direct contact with tannery effluent may cause short-term social memory deficit. While challenging to do, social memory would be an interesting parameter to test in humans employed at tanneries or other occupations with high Cr(VI) exposure (Takahashi et al., 2004). Intriguingly, these few behavior studies reflect some of the neurological effects observed in humans; e.g. social memory loss in patients with failed MoM hip replacements.

**5.1.3 Cr(VI) brain oxidative stress, damage, and redox changes**—Upon entering a cell, Cr(VI) is rapidly reduced to Cr(III) by ascorbate and biological thiols (e.g. glutathione). Along with ephemeral Cr(IV) and (V) species, the reduction also produces ROS such as superoxide anions, hydroxyl radicals and nitric oxide (Bagchi et al., 2001). The role of oxidative stress in neurodegenerative diseases has been thoroughly examined and is amply discussed in other reviews (see Niedzielska et al., 2015; Uttara et al., 2009; Chen et al., 2012). Most Cr(VI) neurotoxicity studies in rodents have measured redox changes, considering both enzymatic [superoxide dismutase (SOD), catalase (CAT)] and non-enzymatic [glutathione (GSH), ascorbate] antioxidants, and measuring malondialdehyde (MDA) levels for oxidative damage, providing a useful panel of biomarkers for comparing toxicity across studies. For a summary of redox data from Cr(VI) neurotoxicity in animal

models literature (Table 4); to simplify comparisons we adjusted the data as percentage of control based on reported data.

Only a few papers have sought to quantify ROS associated with Cr(VI) neurotoxicity by various methods. Singh and Chowdhuri (2018) measured ROS using the fluorescent probe DHE and reported 1.7-fold increased fluorescence in brain ganglia of *Drosophila* after receiving dietary Cr(VI) for 48 h. Travacio et al. (2000) measured brain ROS with the DCFH-dAc reporter and observed 47% increased fluorescence in Swiss mice given 25 mg/kg Cr(VI) in drinking water for 3 days. Mahmoud and El-Twab (2017) reported 1.8-fold increased nitric oxide in rat cortex following 2 mg/kg Cr(VI) i.p. exposure for 30 days. The effects of ROS toxicity in the brain are widespread in neuropathologies, and thought to contribute to protein aggregations, mitochondrial dysfunction, autophagic dysfunction, and neurodegeneration (Tabner et al., 2005; Federico et al., 2012; Dasuri et al., 2013; Singh et al., 2019; Michalska and León, 2020).

Most studies demonstrate decreased non-enzymatic antioxidant levels after Cr(VI) intoxication, primarily considering GSH (Table 4). The relative decreased GSH levels observed across these studies were 15–95% decreased after Cr(VI) exposure. Yet, two papers observed GSH significantly increased following Cr(VI) exposure. One study observed increased GSH in rat cortex (52%) and cerebellum (15%) after Cr(VI) exposure in drinking water (Soudani et al., 2012). While the other instance of Cr(VI)-induced GSH increase (1.33x) occurred in a zebrafish model (Shaw et al., 2020). Another paper reported no effect on rat brain GSH levels after orally administered Cr(VI) (Sarica et al., 2019). Hegazy et al. (2021) observed a dose- and time-dependent decrease in GSH following intra-nasal instillation of potassium dichromate.

The reported effects of Cr(VI) on activity of enzymatic antioxidants [SOD, CAT, and glutathione peroxidase or reductase (GPx or GR)] are much more varied. Three papers reported no effect of Cr(VI) on brain SOD activity in rats and chickens (García-Niño et al., 2015; Iztleuov et al., 2018; Hao et al., 2017) and one paper reported ~50% decreased SOD activity in rats (Mahmoud and El-Twab, 2017). Fatima et al. (2017) conducted the most thorough analysis published to date for SOD activity after Cr(III) nanoparticle exposure in rats for 24 hours, 7 days, or 14 days. After 24 hours, they observed no effect in their low dose (0.5 mg/kg) and ~1.8-fold increased SOD activity in their high dose (2.0 mg/kg), but observed decreased levels at both doses in longer exposures (7–14 days). Two other rodent studies reported elevated SOD activity after Cr(VI) intoxication for 3 or 30 days (Travacio et al., 2000; Nudler et al., 2009). Seven studies considered Cr(VI) effects on CAT activity, with varying effects observed. Two studies in rats showed no effect after 30 days exposure in drinking water or i.p. 24 hours (Nudler et al, 2009; García-Niño et al., 2015). Salama et al. (2016) reported decreased CAT activity after 24 hours intranasal exposure to 1 or 2 mg/kg potassium dichromate, with a greater effect observed after 1 mg/kg (72% and 30% decreased, respectively). Similarly, rats given Cr(VI) for 24 hours via i.p. exhibited a 33% decreased CAT activity (Iztleuov et al., 2018). Conversely, two studies reported increased CAT activity after Cr(VI) exposure. Travacio et al. (2000) reported 74% increased CAT activity in mice given Cr(VI) in drinking water for 3 days. In a study with zebrafish exposed to Cr(VI) for up to 60 days there was a time-dependent increase of CAT activity

(Shaw et al., 2020). Here, the authors observed an initial non-significant decrease of CAT activity after 7 days, then a significant increase after 15, 30 or 60 days (1.58x, 2.58x, 2.35x increase, respectively). Six studies considered Cr(VI) effects on the glutathione peroxidase (GPx) or glutathione reductase (GR) activity, predominantly reporting decreased activity for each. The largest effects observed were a 55% decrease in GR activity in mice exposed to 25 mg/kg orally for 6 weeks and a 40% decrease in GPx in rats given i.p. 2 mg/kg Cr(VI) for 30 days (Abu Zeid et al., 2018; Mahmoud and El-Twab, 2017). Only one study reported an increase in either; Nudler et al. (2009) reported 2-fold increased GR activity in hypothalamus of rats receiving 100 ppm Cr(VI) for 30 days.

Thus far, every study that has considered brain MDA levels after Cr(VI) exposure has reported elevated levels following exposure (Table 4). MDA is an end product of lipid peroxidation and often elevated in peripheral blood of patients with neurodegenerative diseases (Dib et al., 2002; Greilberger et al., 2008; Ozcankaya and Delibas, 2002). Importantly, MDA can have detrimental effects on neuronal mitochondrial respiration, targeting mitochondrial complexes I, II, and V and promote formation of ROS (Long et al., 2009). The highest reported Cr(VI)-induced MDA levels in a vertebrate model were from Hegazy et al. (2021). Using a rat model exposed to 0.5 mg Cr/kg/d, 5 d/week for two months, they observed a dose- and time-dependent increase in rat brain MDA levels that peaked at two months with 127.24 nmol MDA/g tissue (8.21x increased MDA). Concomitantly, 14 days also exhibited the greatest reduction in GSH levels, which were decreased by ~59%. Based on the reported data, MDA currently serves as the best biomarker to compare Cr(VI)-induced oxidative damage in rodent brain tissues and is consistently elevated upon exposure. Whereas antioxidant responses to Cr(VI) intoxication are variable across studies, possibly due to the sensitivity of redox balance or gender and age differences in brain ascorbic acid levels (Kume-Kick and Rice, 1998).

**5.1.3 Cr(VI) Neuropathology**—Nine papers reported histopathological assessments in their Cr(VI) neurotoxicity studies; only one reported a lack of histopathological effects in rats (García-Niño et al., 2015). Soudani et al. (2012) were the only to report tumor formation, an oligodendroglioma in a female rat. Widespread neurodegeneration was observed in multiple studies across multiple species, with notable toxicity to Purkinje cells of the cerebellum (Soudani et al., 2012; Fahmy, 2017; Hao et al., 2017). Hegazy et al. (2021) observed a dose- and time-dependent significant decrease in the number of viable neurons in cortical gray matter, with increased reactive astrogliosis. The authors noted GFAP<sup>+</sup> astrocytes were significantly increased in number and characterized by cellular hypertrophy. Gliosis and satellitosis were also frequently observed. However, these effects were most likely responses to neurodegenerative effects and not due to direct Cr(VI)-induced activation, as these were typically observed at the highest doses and durations tested and were often associated with neurodegeneration. Other neurodegenerative changes including hemorrhage, neuronal vacuolation, edema, and neuronophagia were reported after Cr(VI) intoxication (Mathur et al., 1977; Soudani et al., 2012; Salama et al., 2016; Fahmy, 2017; Fatima et al., 2017; Hao et al., 2017; Abu Zeid et al., 2018; Shaw et al., 2020). Currently it appears Cr(VI) has a widespread neurotoxic effect with no clear evidence of

regional or phenotypic specificity, but more detailed studies are needed to evaluate this further.

**5.1.4 Other Endpoints Considered for Cr(VI) Neurotoxicity**—A few other points are worth discussing from the available Cr(VI) animal neurotoxicity studies: effects on inflammation, acetylcholinesterase, and trace element homeostasis.

Three papers measured neuroinflammation and found elevated pro-inflammatory cytokines following Cr(VI) exposure in rats (Salama et al., 2016; Mahmoud and El-Twab, 2017; Hegazy et al., 2021). Salama et al. (2016) reported a dose-response increase in brain IL-1 $\beta$  and phospho-PKB after 0.5, 1.0, or 2.0 mg/kg Cr(VI) exposure, but they attributed the ~4x increased levels for both cytokines to widespread necrosis that was evident at the highest Cr(VI) concentration. Mahmoud and El-Twab (2017) reported ~3- and ~4.7-fold increased TNF- $\alpha$  and IL-6 following daily 2 mg/kg i.p. injection of Cr(VI) for 30 days. Hegazy et al. (2021) observed dose- and time-dependent increases in IL-1 $\beta$ , PI3K, and PKB following intra-nasal instillation of potassium dichromate.

Four studies considered Cr(VI) effects on brain acetylcholinesterase, a key enzyme for clearing the neurotransmitter acetylcholine from synapses. Importantly, alterations to the cholinergic system have been observed in Alzheimer's disease and disrupted REM sleep, an early non-motor symptom of Parkinson's disease (Francis et al., 1999; Platt and Riedel, 2011). In all cases, studies showed Cr(VI) decreased acetylcholinesterase activity by at least 40% (Soudani et al., 2012; Kim and Kang, 2016; Mahmoud and El-Twab, 2017; Abu Zeid et al., 2018).

Several studies considered changes in expression of stress response genes. Shaw et al. (2020) observed upregulation of stress response genes *Nrf2*, *Nqo1*, *HO-1*, and *Ucp2* as well as apoptotic genes *p53*, *Bax*, *Caspase-9*, and *Caspase-3* after 60 days Cr(VI) exposure in zebrafish. Nudler et al. (2009) reported elevated levels of metallothionein isoform 3 (*MT3*) in hypothalamus, of metallothionein isoform 1 (*MT1*) in anterior pituitary gland, and *HO-1* in both regions of rats after 30 days exposure to 100 ppm Cr(VI) in drinking water. Importantly, they observed no effect on these genes in the liver, suggesting the brain is more vulnerable than the liver to Cr(VI) in drinking water. Mahmoud and El-Twab (2017) observed elevated levels of JAK-STAT genes *JAK1* and *STAT3*, along with elevated *SOCS3* in cerebrum of rats after 30 days daily i.p. 2 mg/kg Cr(VI). They suggested the *SOCS3* elevation was in response to the JAK-STAT induction, and these effects were associated with the ongoing neuroinflammation. Abu-Zeid et al. (2018) also observed chronic Cr(VI)-induced elevated *p51*, *Bax*, and *Caspase-3* in rats.

As a measure of Cr(VI)-induced DNA damage, Abu-Zeid et al. (2018) observed approximately 2-fold increased 8-hydroxy-2'-deoxyguanosine (8-Oxo-dG) in brain homogenates of rats exposed to Cr(VI) orally for 6 weeks.

Zhu et al. (2018) assessed trace element homeostasis in chickens following 14, 28, or 42 days of potassium dichromate exposure. Their results showed levels of calcium, copper,

zinc, and magnesium increased following Cr(VI) exposure, while manganese and iron levels decreased with Cr(VI) exposure doses and time.

Singh and Chowdhuri (2017) utilized reporter strains of fruit flies to assess Cr(VI)-induced toxicity across neuronal subtypes (cholinergic or dopaminergic) and in glial cells of brain ganglia. They showed a dose- and time-dependent decrease in fluorescence intensity for flies with a pan-neuronal marker (*ELAV-GAL4*), but no change in fluorescence intensity for flies with a glial cell marker (*Repo-GAL4*). Fly strains with cholinergic neurons labeled (*ChAT-GAL3*) exhibited a dose- and time-dependent decrease in fluorescence intensity, while dopaminergic neurons (*Ddc-GAL4*) only exhibited a decrease in neuron count at the highest and longest Cr(VI) exposure studied. These observations were supported by their Annexin V staining, demonstrating significantly elevated apoptosis in their highest dose (20 µg/mL) and longest duration (48 h).

**5.1.5 Summary of Neurotoxicity Observed in Animal Models**—It is clear from these *in vivo* studies that Cr(VI) can accumulate in the brain and induce histological and biochemical damage. Impaired locomotor and memory functions were observed in multiple studies following Cr(VI) exposure. Oxidative damage, AChE levels, and neurodegeneration have been most often considered for Cr(VI) neurotoxic endpoints. Some studies have shown elevated pro-inflammatory cytokines in response to Cr(VI). Much work remains to determine what regions or cell types are most vulnerable, and what neurobehavioral deficits might arise.

## 5.2 Cell culture work

Three different groups have reported Cr(VI) neurotoxicity studies in cell culture, using concentrations ranging from 0.1–100 µM Cr(VI) for up to 72 h; the experimental details are summarized in Table 5. Quinteros et al. (2007, 2008) observed different sensitivities to Cr(VI) for different neuronal subtypes (lactotrophs and gonadotrophs) within the anterior pituitary. Using primary rat neuronal cultures, they demonstrated Cr(VI) induced ROS- and caspase-3-mediated apoptosis more severely in lactotrophs than gonadotrophs. Dashti et al. (2014) exposed mouse primary cerebellar granule cells (CGCs) at various stages of neuronal maturation, and identified immature CGCs were significantly more sensitive than cells undergoing maturation or fully matured. Subsequently, this same group observed elevated ROS production (DCFH-DA) and lipid peroxidation (MDA) in PC12 cells exposed to potassium dichromate for 24, 48, or 72 hours and reported IC<sub>50</sub> values of 22.02, 1.88, and 1.85 µM, respectively. Altogether, these studies agree Cr(VI) induces ROS-mediated apoptosis in a concentration-dependent manner, but the impact of exposure duration was inconsistent. These studies assert the need for further *in vivo* investigation into Cr(VI) neurotoxicology to assess sensitivities to neuronal subtypes and toxicity across neurodevelopment. Already we can see a difference in Cr(VI) sensitivity between lactotrophs and gonadotrophs in the hypothalamus and different effects depending on neuron maturity (Quinteros et al. 2007, 2008).

### 5.3 Potential prevention and interventional studies

Several studies have considered antioxidant co-administration to alleviate Cr(VI) neurotoxicity. Selenium has a well-established role for quenching ROS in the brain (see review by Solovyev, 2015), and has been considered in three separate Cr(VI) neurotoxicity studies using rat, chicken, and guinea pig models (Soudani et al., 2012; Hao et al., 2017; Fahmy, 2017). In all three studies selenium co-treatment reduced neuronal loss, and in the rat study appeared to alleviate Cr(VI)-induced oxidative damage and pathology. The guinea pig study also observed an increase in the astrocyte number following Cr(VI), which was decreased by co-administration of selenium and interpreted as decreased astrocyte activation (Fahmy, 2017). These observations suggest selenium prevents ROS-mediated neurodegeneration, alleviating subsequent astrocyte activation. More recently, boron has received similar consideration as an antioxidant supplement (see review by Pizzorno, 2015). When co-treated with Cr(VI), two studies in rats showed boron attenuated Cr(VI)-induced oxidative damage and endogenous antioxidant depletion (Sarica et al., 2019; Iztleuov et al., 2018). Ascorbic acid is widely known for attenuating Cr(VI) toxicity and is well documented in the rodent brain, so it is surprising only one study has considered the effects of ascorbic acid on Cr(VI) neurotoxicity (Standeven et al., 1992; Wise et al., 1993; Xie et al., 2004; Kume-Kick and Rice, 1998). Abu Zeid et al. (2018) conducted a rat study involving groups given ascorbic acid for six weeks as either co-treatment (120 mg/kg ascorbate and 25 mg/kg potassium chromate co-administered) or pre- and co-treatment (ascorbate treatment started 2 weeks prior to chromate, and continued for all six weeks). The groups receiving ascorbic acid were found to have: (1) reduced oxidative damage, measured by levels of MDA, protein carbonylation, and 8-oxo-2'-deoxyguanosine, (2) reduced apoptotic markers p-53, Bax and caspase-3, (3) restored endogenous antioxidant enzymes and AChE levels, and (4) alleviated pathological effects (e.g. neuronal vacuolation). Other phytochemicals have been considered for alleviating Cr(VI) neurotoxicity, including caffeic acid phenethyl ester, rosmarinic acid, and curcumin (Mahmoud and El-Twab, 2017; Dashti et al., 2014; García-Niño et al., 2015). In each case, the antioxidant was shown to attenuate Cr(VI) toxicity, but whether this effect was from simply preventing Cr(VI) uptake was not determined in these studies. Singh and Chowdhuri (2018) considered a genetic approach to alleviate Cr(VI) neurotoxicity using a *Drosophila* model and modulating expression of the oxidative stress response gene *sestrin*. The authors found *sestrin* overexpression protected neurons from Cr(VI)-induced death by upregulating autophagy and reducing ROS levels, whereas *sestrin* knockdown increased neuronal vulnerability to Cr(VI). These studies demonstrate a possible pharmacological intervention for individuals exposed to Cr(VI), such as patients with failed MoM hip replacements; especially since most of these supplements are already commercially available.

## 6. Current Limitations and New Insights into Cr(VI) Neurotoxicology

Collectively, from human, animal, and cell culture studies it is clear that Cr(VI) is a sinister neurotoxicant that can affect behavior and health in subtle ways. These observations are generally summed up in Figure 2. While Cr(VI) is a known human carcinogen and poses a significant occupational health hazard, there is little evidence that Cr(VI) has a significant

contribution to brain cancer, as two meta-analyses of the current literature failed to find an association (Cole and Rodu, 2005; Deng et al., 2019).

There is growing evidence suggesting Cr(VI) can have a variety of effects on neurobehavior. Various studies in human populations exposed to Cr(VI) exhibited social memory loss, depression, polyneuropathy, olfactory dysfunction, and neuropsychological impairments. These effects are particularly alarming for children and elderly individuals, who seem to be more susceptible to Cr(VI) behavioral effects. Importantly, it seems that neurons are more susceptible to Cr(VI) toxicity during neurodevelopment, as demonstrated in the child neurodevelopment study in Spain, the *in utero* exposure assessment for autism spectrum disorder, and the study using primary cerebellar granule cells (Caparros-Gonzalez et al., 2019; Dashti et al., 2014; Talbott et al., 2015). However, there is little to no neurotoxicology data investigating how age affects neurotoxic outcome of Cr(VI). Perhaps in children this vulnerability is due to the mitotic activity of a developing brain, as Cr(VI) is a well-defined clastogen. Whereas geriatric vulnerability may be due to clastogenic toxicity combined with life-long accumulation of neurodegenerative endpoints (e.g. protein aggregation, DNA damage accumulation, persistent neuroinflammation, weaker BBB). Data considering Cr(VI) exposure and neurologic morbidity in children and geriatrics would greatly help the Cr(VI) neurotoxicology field define the scope of Cr(VI) neurotoxic potential.

From the collection of animal studies available, it is clear that Cr(VI) is more neurotoxic via inhalation than i.p. injection or drinking water. Salama et al. (2016) demonstrated this with a comparison of i.p. versus intra-nasal administered Cr(VI) for 24 h, showing similar neuropathology with a much lower dose administered intra-nasally. However, i.p. injection does not adequately reflect a real life exposure, and lacking data for blood Cr in these models limits their translatability to occupational or environmental exposure settings. The impact of exposure route is also apparent when comparing across studies using similar concentrations and durations; for example, intra-nasal 0.5, 1.0 or 2.0 mg Cr/kg induced a greater fold increase in MDA than 15 mg Cr/kg i.p. as observed between Salama et al.'s study and others (Table 4). As is the case for most metals, Cr(VI) typically exhibited a dose-response effect for inducing ROS and oxidative damage in the brain. Interestingly, though, the duration of Cr(VI) exposure seemed to have little effect (Bagchi et al., 2002; Dashti et al., 2014). Perhaps the most limiting factors for understanding Cr(VI) neurotoxicity are: 1) the lack of recognizing what brain regions are most vulnerable to Cr(VI), and 2) the lack of recognizing which cell types are most vulnerable to Cr(VI).

Cr(VI) is most often encountered with other metals, but such metal mixtures have not been thoroughly considered. Two studies have considered the neurotoxicity of Cr in combination with other metals. In one study investigating neurotoxicity of welding fumes, rats were given an intratracheal dose of: (i) 2 mg/kg manganese nanoparticles in combination with (ii) 2 mg/kg chromium hydroxide nanoparticles, (iii) 2 mg/kg iron nanoparticles, or (iv) both, once a day, five times a week for four weeks (Máté et al., 2017); the design of this study was to use doses that replicate typical daily exposures for welders. Electroencephalogram readings shifted to higher frequencies and lengthened evoked potential latency following manganese nanoparticle exposure. These effects were strongly diminished by iron, but unaffected by Cr nanoparticles. In the other study, human neuroblastoma cells (SK-N-SH) were exposed to

a combination of arsenic, Cr, and copper leached from chromated copper arsenate-treated wood (Hu et al., 2013). Here, the authors noted arsenic neurotoxicity was inhibited by Cr(III), suggesting a potential neuro-pharmacological role for the Cr(III).

## 7. Conclusions and Future Directions for Cr(VI) Neurotoxicity Studies

The neurotoxicity of Cr(VI) has been considered across a wide variety of species, including humans, rodents, chicken, fish, birds, and flies. The results largely describe to Cr(VI) induction of oxidative stress and oxidative damage, though the implications on long-term brain health and neurobehavior is unknown. Pathologic effects of Cr(VI) on the brain are likely to be regionally specific due to regional differences in GSH and ascorbic acid levels, reducers of Cr(VI) and oxidative damage, and differing vulnerability observed between gonadotrophs and lactotrophs in the anterior pituitary.

### 7.1 Cr(VI) Entry to the Brain

It's clear that Cr(VI) can induce widespread oxidative damage, but the effects of that damage have yet to be fully understood, especially in the brain. This widespread effect may indicate Cr(VI) can cross the BBB or, alternatively, target the BBB.

### 7.2 Cr(VI) and Considerations for Age

We need a better understanding of how Cr(VI) affects neurodevelopment; we can see it is more toxic to immature neurons, exposed children may be neurodevelopmentally challenged, and exposed pregnant mothers have an increased risk of their child developing autism. On the flip side of the age spectrum, little is known how Cr(VI) might affect the brain in an aged individual, where Cr might pass the BBB more easily or might have bioaccumulated to a hazardous level in the brain. One study measured normal Cr levels, without any exposure, in an aging mouse model, observing a non-significant 19.9% decrease in Cr content (Massie et al., 1983). This outcome was a similar trend to Cr levels measured in CSF from Parkinson's disease patients, begging the question if Cr has an essential role in some brain regions (Bocca et al., 2004; Alimonti et al., 2007). Studies have also shown Cr(VI) induces cellular senescence in bronchial fibroblasts, germ cells, and hepatocytes, suggesting a potential role as a gerontogen, i.e. a chemical that contributes to or accelerates the aging process (Zhang et al., 2016; Sivakumar et al., 2014; Val et al., 2015; Sorrentino et al., 2014). Indeed, a case-control study of welders exposed to Cr(VI) found blood Cr levels to be the main determinant for elevated levels of senescence biomarkers in blood samples, which replicated the observations from their earlier *in vitro* and *in vivo* studies (Alexopoulos et al., 2008; Katsiki et al., 2004). Hence, we need to understand more about how Cr(VI) might affect brain aging and an aged brain, which can inform us more about how exposures may contribute to age-related diseases.

### 7.3 Cr(VI) and Brain Cell Target

Thus far no studies have compared how Cr(VI) affects different types of brain cells, and most cell types in the brain have yet to be considered. There are at least 8 major cell types in the brain, including neurons, astrocytes, microglia, ependymal cells, pericytes, and brain endothelial cells. Most studies have not considered which cell types are responsible for

oxidative damage observed in the brain following Cr(VI) exposure, and the implications for each cell type are strikingly different. Studies with homologous cell types in other tissues show the importance of this distinction. A study from Cao et al. (2020) reported Cr(VI) effects on human umbilical vein endothelial cells (HUVECs) and THP-1 cells (monocytes) increased expression of ICAM-1 and VCAM-1, and enhanced the adhesion of THP-1 cells to HUVECs. These results are useful to inform future Cr(VI) neurotoxicity studies investigating effects on brain vascular endothelial cells, and suggest Cr(VI) may affect endothelial cells to recruit peripheral monocytes/macrophages, inducing inflammatory effects. This study also reported Cr(VI)-induction of NF- $\kappa$ B, cleaved caspase-1, and IL-1 $\beta$  in THP-1 cells, which may be translatable to homologous microglial cells in the brain. Given the often observed “widespread neurodegeneration” in Cr(VI) animal studies, it would be informative to determine Cr(VI) effects on cells of the neurovascular unit and BBB. Results from such a study would further aid in identifying other potential neurological endpoints to consider in Cr(VI)-exposed human populations.

#### 7.4 Cr(VI) Neurotoxic Mechanism

Thus far no studies have determined a mechanism for Cr(VI) neurotoxicity. Toxic effects have been pinpointed, such as lipid peroxidation, induction of apoptosis, altered acetylcholinesterase, and changes in neuroinflammatory markers – but no studies have pieced together a mechanism for Cr(VI)’s toxic effects in the brain or in any particular type of brain cells. Figure 3 summarizes the mechanistic data currently available for Cr(VI) neurotoxicology. In other organs and cell types, the mechanisms of Cr(VI) toxicity are much better developed and can provide useful insights for future Cr(VI) neurotoxicology studies (for reviews, see Holmes et al., 2008, Wise et al., 2008, Saha et al., 2011; DesMarias and Costa, 2019). Briefly, Cr(VI) is well characterized to induce ROS in a Fenton-like reaction (Shi and Dalal, 1990; Ding and Shi, 2002). Further details into Cr(VI)-mediated oxidative damage or damage from its resulting ROS may help inform neurodegenerative diseases affected by ROS-driven mechanisms. Cr(VI) is reduced in cells, ultimately to Cr(III) with Cr(V) and Cr(VI) produced during the reduction and these species rival ROS in mechanisms in other organs, but the role of these species is unknown in the brain. Epigenetics play an important role in neurodevelopment and brain organization. Several studies in other cell types illustrate Cr(VI) can disrupt methylation status of multiple genes in a variety of species and can increase expression of histone methyltransferases, key enzymes in modulating methylation status (Sun et al., 2009; Chen et al., 2016; Wang et al., 2018; Lv et al., 2018; Feng et al., 2018). Mitochondrial health is critical to neuronal health, given their high demand for ATP to maintain their polarized state, and many neurodegenerative diseases exhibit impaired mitochondrial dynamics. Recently there has been a resurgence of investigations into Cr(VI) and mitochondrial effects in other cell types, with many reporting altered expression of mitochondrial proteins, altered calcium signaling, and impaired mitochondrial respiration and ATP production (Xiao et al., 2012; Wise et al., 2018; Zhang et al., 2021; Yang et al., 2021). In sum of this review, it is clear Cr(VI) is neurotoxic, but there is much to be discovered.

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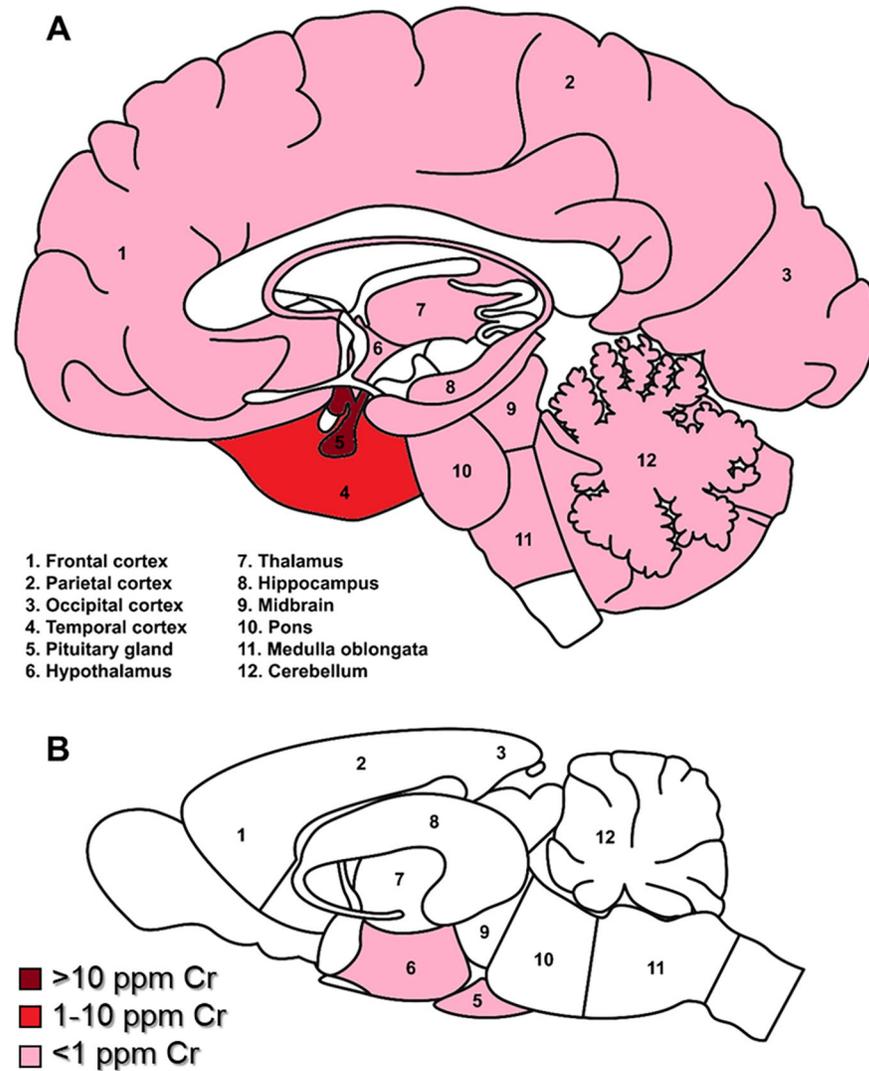
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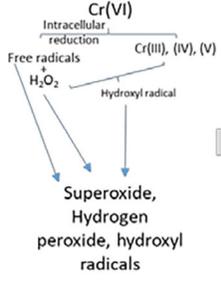
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**Figure 1. Relative distribution of reported Cr deposition in human (A) and rodent (B) brains.** Dark red indicates Cr levels greater than 100  $\mu\text{g/g}$ ; red = 10–100  $\mu\text{g/g}$ ; pink indicates less than 1  $\mu\text{g/g}$ ; white areas indicate no data reported. NB: most rodent studies measured whole brain Cr, thus there is limited data for regional Cr deposition.

Cr-mediated ROS	Key signaling pathways	Cellular & pathological responses	Phenotypes Behavior changes
 <p>Cr(VI) undergoes intracellular reduction to Cr(III), (IV), (V), producing free radicals, H<sub>2</sub>O<sub>2</sub>, and hydroxyl radicals. These lead to superoxide, hydrogen peroxide, and hydroxyl radicals.</p>	<ul style="list-style-type: none"> <li>• Lipid peroxidation</li> <li>• Acetylcholinesterase inhibition</li> <li>• TNF-α and IL-6</li> <li>• Caspase-3 apoptosis</li> </ul>	<ul style="list-style-type: none"> <li>• Axonal degeneration</li> <li>• Astrocyte activation</li> <li>• Regional loss of gray matter</li> <li>• Purkinje cell loss in cerebellum</li> <li>• Hemorrhage and edema</li> <li>• Neuronophagia</li> <li>• Inflammation</li> <li>• Essential element dyshomeostasis</li> </ul>	<ul style="list-style-type: none"> <li>• Painful dysesthesia</li> <li>• Depressed mood</li> <li>• Short-term memory deficit</li> <li>• Location disorientation</li> <li>• Polyneuropathy</li> <li>• Olfactory dysfunction</li> <li>• Motor neuron disease</li> <li>• Impaired child attention development</li> <li>• Autism</li> <li>• Loss of social memory</li> <li>• Decreased motor activity</li> </ul>

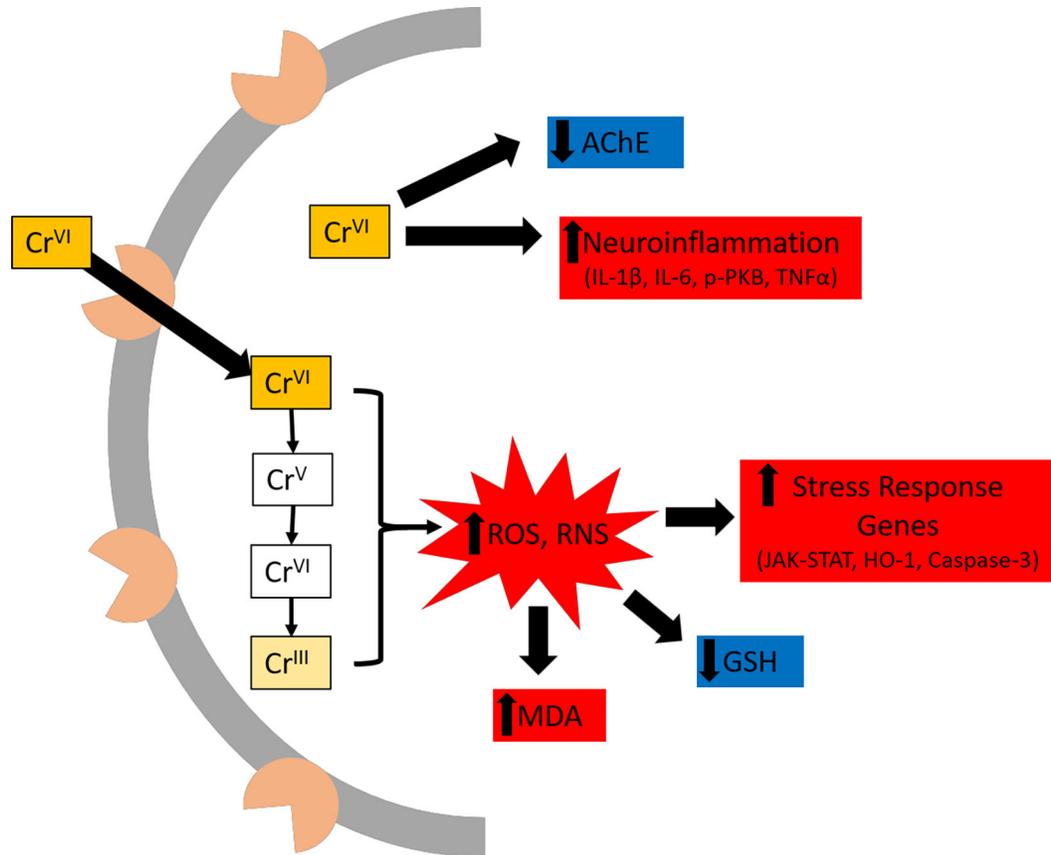
**Figure 2. Summary of key findings from Cr(VI) neurotoxicity literature.**

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**Figure 3. Schematic of early events in Cr(VI) neurotoxicity.**

Cr(VI) enters a cell through phosphate or sulfate channels. In the cytoplasm, it is rapidly reduced to Cr(III), which also produces ROS. Oxidative stress and oxidative damage were most often reported as decreased GSH and increased MDA, while several studies have reported increased mRNA of stress response genes often induced by ROS. Cr(VI) increases pro-inflammatory cytokines (e.g. IL-1 $\beta$ , IL-6, TNF $\alpha$ ) and decreases AChE levels, though it is currently unclear by what mechanism(s). Gray semi-circle represents cell membrane, with pink shapes to represent sulphate/phosphate channels.

**Table 1.**

**Cr Levels Reported in Human Brain and CSF**

Brain Region	Age (n)	Cr Concentration (µg/g)	Quantification Method	Brain Health Status	Reference
Whole Brain	N.R. (10)	0.01	SSMS	Healthy	Goody et al., 1975
	N.R. (2)	0.03		Healthy	
		0.09			
Basal Ganglia	5 h – 74 y (8)	Range = 0.648–4.088 *	INAA	Healthy	Höck et al., 1975
Cerebral Cortex		Range = 1.315–48.818 *	INAA	Healthy	Kanabrocki et al., 1975
		Detected in 4/14, by age: 54 = 160.6 * 58 = 203.03 * 63 = 100.0 * 72 = 251.15 *			
Pituitary	42 – 83 (14)		INAA	Healthy	
Brain	0 – 1 (7)	Range = 0.242–1.273 *	INAA	Healthy	Persigehl et al., 1977
	20 – 40 (6)	<0.606 *			
	50 – 83 (6)	Range = 0.606–2.909 *			
Brain	Adults (28)	0.127 ± 0.013	INAA	Healthy	Markesbery et al., 1984
	Infants (7)	Range = <0.006–0.602 0.067 ± 0.015			
Cerebellar hemisphere	Adults (28)	0.265			
Cerebellar vermis		0.260			
Putamen		0.260			
Caudate nucleus		0.0520			
Hippocampus		0.0807			
Cerebral cortex (L hemisphere)	Age-matched (23)	2.42 ± 1.61 *			
Astrocytoma	28 – 64 Mean = 45 (15)	1.76 ± 1.67 *		Cancer	
Frontal cerebrum	N.R. (8)	0.15 ± 0.05	ICP-AES	Healthy	Rajan et al., 1997
Temporal cerebrum		0.18 ± 0.12			
Parietal cerebrum		0.16 ± 0.09			
Somatosensory cortex		0.13 ± 0.07			

Brain Region	Age (n)	Cr Concentration (µg/g)	Quantification Method	Brain Health Status	Reference
Occipital cerebrum		0.14 ± 0.08			
Cerebellum		0.11 ± 0.06			
Mid-brain		0.12 ± 0.06			
Pons		0.13 ± 0.04			
Hypothalamus		0.11 ± 0.05			
Thalamus		0.14 ± 0.09			
Hippocampus		0.10 ± 0.08			
Medulla oblongata		0.12 ± 0.06			
Frontal Cortex	33.06 ± 4.8 (59)	701 ± 113 *	ICP-MS	Low-pollution cities	Calderón-Garcidueñas et al., 2013
		1210 ± 84 *		Mexico city (high pollution)	
Temporal cortex	30 (1)	1.36 *	INAA	Healthy	Civit et al., 2000
Oligodendrogloma	38 (1)	1.12 *		Cancer	
Meningioma	64 (1)	<0.242 *		Cancer	
CSF	63.8 ± 13.7 (13)	1.39 ± 0.64 ng/mL	SF-ICP-MS	Control	Bocca et al., 2004
	64.9 ± 10.8 (26)	0.60 ± 0.47 ng/mL		Parkinson's disease	
CSF	66.2 ± 14.7 (20)	1.28 ± 0.59 ng/mL	SF-ICP-MS	Control	Alimonti et al., 2007
	64.5 ± 10.6 (42)	0.65 ± 0.46 ng/mL		Parkinson's disease	
CSF	63 (252)	5–24 nmol/L (0.26–1.25 ng/mL)	ICP-MS	Control	Harrison-Brown et al., 2020
	65 (141)	6–36 nmol/L (0.31–1.87 ng/mL)		Well-function implant	
	64 (68)	7–32 nmol/L (0.36–1.66 ng/mL)		Hip Revision	

N.R. = Not Reported; SSMS = Spark-Source Mass Spectrometry; INAA = Instrumental Neutron Activation Assay; ICP-AES = Inductively Coupled Plasma-Atomic Emission Spectroscopy; SF-ICP-MS = Sector Field-Inductively Coupled Plasma-Mass Spectrometry

\* Cr values adjusted from dry weight to wet weight by adjusting for 77% brain water content, according to Keep et al., 2013

Table 2.

Summary of Cr (VI) Neurological Studies in Humans

Occupation/Exposure	Geographic Region	Date(s) of Exposure	Number People Studied	Measured Cr(VI) Exposure	Health Effect(s)	Calculated Risk	Reference
<i>Cancer Studies</i>							
Ferroalloy and Stainless Steel Production	France	1 year, 1952–1982	2,269	N.R.	Brain cancer	SMR = 3.36 (0.41–12.24)	Moulin et al., 1990
Arc Welder	Germany	6+ months, 1950–1970	2,416	N.R.	Brain cancer	2.7 (0.2–29.3) RR	Becker et al., 1991
*Chromium and compounds*	Denver, CO	1976–1983	252 cases 222 controls	N.R.	Brain cancer	Father's OR = 0.8 (0.3–2.8)	Feingold et al., 1991
Boiler welder	Norway	1942–1981	2,597	N.R.	Brain cancer	SIR = 102 (49–188)	Danielson et al., 1996
Chrome tannery worker	Italy	6+ months, 1955–1988	1,244	N.R.	Brain cancer	SMR = 168 (46–431)	Montanaro et al., 1997
Concrete worker	Sweden	1971–1986	33,503	N.R.	Brain cancer	SIR = 107 (88–129)	Knutsson et al., 2000
Turner, toolmaker	Finland	N.R.	413,877	12 µg/g/m <sup>3</sup> chromates	Brain cancer	SIR = 11.9	Wesseling et al., 2002
Occupational	All (meta-analysis)	N.R.	(Meta-analysis)	N.R.	Brain cancer	SMR = 88 (64–118)	Cole and Rodu, 2005
Chromium salts	Canada	N.R.	21,020 cases 5,039 controls	N.R.	Brain cancer	Multivariable OR: Men = 1.40 (0.74–2.63) Women = 1.06 (0.22–5.12) Both = 1.35 (0.75–2.41)	Pan et al., 2005
Cr Plating	Japan	N.R.	1,193	N.R.	Brain cancer	SMR = 9.14 (1.81–22.09)	Hara et al., 2010
Cement Production	Korea	1988–2007 > 1 day	10,740	N.R.	Brain cancer	3 cases SMR = 3.58 (0.74–10.47)	Koh et al., 2011
Aircraft Manufacturing Worker	Burbank, CA	> 1 year, 1960–1996	77,943	7,458 exposed workers (16.5%)	Brain/CNS cancer	Chromates SMR = 0.88 (0.54–1.36) Non-factory SMR = 1.09 (0.90–1.32)	Lipworth et al., 2011
Jet Fuel Manufacturing	Connecticut, USA	1952–2004	210,784	Mean = 0.2 µg/g/m <sup>3</sup>	Glioblastoma	OR: >0–0.065 = 1.00 (0.55–1.68) 0.066–0.093 = 0.93 (0.56–1.50) 0.094+ = 0.81 (0.53–1.22)	Marsh et al., 2013
Jet Fuel Manufacturing	Connecticut, USA	1952–2004	113,439	Mean = 0.24 µg/g/m <sup>3</sup>	Glioblastoma	RR: >0–0.065 = 1.17 (0.79–1.72) 0.066–0.093 = 0.75 (0.54–	

Occupation/Exposure	Geographic Region	Date(s) of Exposure	Number People Studied	Measured Cr(VI) Exposure	Health Effect(s)	Calculated Risk	Reference
						1.04 0.094+ = 0.90 (0.67–1.23)	
Ferromagnesium and stainless steel production	Finland	1967–2004	8,146	22–143 µg/g/m <sup>3</sup>	Brain cancer	SIR: 0.93 (0.55–1.47)	Huvinen and Pukkula, 2013
Welding	7 countries (mostly UK & Germany)	N.R.	1,800 cases 5,160 controls	N.R.	Glioma	No significant effect detected	Parent et al., 2017
Occupational	All (meta-analysis)	N.R.	(Meta-analysis)	N.R.	Cancer	CNS cancer = 1.22 (0.67–2.23) Brain cancer = 1.04 (0.87–1.24)	Deng et al., 2019
<i>Metal-on-Metal Hip Replacement Studies</i>							
Failed Metal-on-Metal Hip Replacement	Japan	2 years	1	22.1 µg/g/L	Polynuropathy	N.R.	Ikeda et al., 2010
Failed Metal-on-Metal Hip Replacement	United Kingdom	8 ± 1.7 years	25	Blood = 1.42 (1.01–3.86) µg/g/L	Benign essential tremor; regional decreased gray matter	N.R.	Clark et al., 2014
Failed Metal-on-Metal Hip Replacement	United Kingdom	4.4 years	10	Blood = 338 nmol/L (144–164)	Depression, short term memory deficit, neurocognitive deficit	N.R.	Green et al., 2017
Failed Non-Metal-on-Metal Hip Replacement	Netherlands	6 months, fatal	1	Blood = 48.8 µg/L	Asymmetrical hearing loss, visual impairment, unintentional weight loss	N.R.	Peters et al., 2017
Failed Metal-on-Metal Hip Replacement	France	<1 year	1	Blood = 65.9 µg/L	Rapid cognitive decline, behavioral disorders, lack of memory, brutal hearing and visual loss	N.R.	Leccoanet et al., 2019
<i>Other Health Endpoints</i>							
Chromate factory worker	Japan	7+ years post-employment	33	N.R.	Impaired olfaction, perforated septum (unrelated to olfaction loss)	N.R.	Watanabe and Fukuchi, 1981
Chromium Production Worker	Baltimore, MD	N.R.	2,354	N.R.	Cause of death: Mental & psych disorders; Nervous system disorder	SMR: 1.42 (1.00–1.95) 0.53 (0.32–0.83)	Gibb et al., 2015
Cr Plating	Korea	0.9–18.2 years (avg = 7.9)	N.R.	Cr(III) = 0.059, 0.021, 0.0063, 0.0047 mg/m <sup>3</sup> Cr(VI) = 0.013, 0.033, 0.0054, 0.0047 mg/m <sup>3</sup>	Impaired olfactory recognition, but detection unaffected	N.R.	Kitamura et al., 2003

Occupation/ Exposure	Geographic Region	Date(s) of Exposure	Number People Studied	Measured Cr(VI) Exposure	Health Effect(s)	Calculated Risk	Reference
River Pollution	N Spain	Lifetime	9,434	N.R.	Motor Neuron Disease	SMR = 1.12 (1.08–1.17) 15.7% incr risk	Sánchez-Díaz et al, 2018
Environmental Pollution	S Spain	Lifetime (age 6–11, avg 7.8)	393	0.44 (SD±1.32) µg/g L urine 0.93 (SD±2.45) µg/g creat 0.33 (SD±1.13) µg/g hair	Impaired neuropsychological development	N.R.	Caparros-Gonzalez et al., 2019

N.R. = Not reported; SMR = standard mortality ratio; SIR = standard incidence ratio; OR = odds ratio; RR = relative risk

Table 3.

Animal Models in Cr (VI) Neurotoxicology Studies

Species (Gender)	Cr Compound	Exposure Route	Dose(s)	Duration	Cr Level Reported (ug/g)	Method of Detection	Reference
Rabbit (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	2 mg/kg	3 and 6 weeks	1.6 and 1.3	Not reported	Mathur et al., 1977
Wistar rat (M)	Na <sub>2</sub> CrO <sub>4</sub>	d.w.	700 mg/L	28 d	N/A		Diaz-Mayans et al., 1986
			70 mg/L	7 d	N/A		
Swiss mice (F)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	2 mg/kg	24 h	N/A		Travacio et al., 2000
		d.w.	25 mg/kg	1-3 d	0.5	AAS	
C57BL/6Ntac (F)	Na <sub>2</sub> CrO <sub>4</sub>	Oral	95 mg/kg	24 h	N/A		Bagchi et al., 2001
C57BL/6TSG p53 (M) (p53 deficient)							
Albino mice (M) (ddY strain)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	20 mg/kg	15 min, 3 h, 24 h	0.28 ± 0.13, 0.31 ± 0.27, 0.15 ± 0.03	AAS	Ueno et al., 2001
C57BL/6Ntac (F)	Na <sub>2</sub> CrO <sub>4</sub>	Oral	1.9, 19, 95 mg/kg	0, 12, 24, 48, 72, 96 h	N/A		Bagchi et al., 2002
Wistar rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	d.w.	500 ppm = 73.05 mg Cr/kg/d	30 d	Pituitary = 0.64 Hypothalamus = 0.059	AAS	Quinteros et al., 2007
Wistar rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	d.w.	100 ppm = 11.6 mg Cr/kg/d	30 d	Pituitary = 0.274 Hypothalamus = 0.026	AAS	Nudler et al., 2009
Swiss albino mice (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	8.0 mg/kg	12h	1.40	ICP-MS	Döker et al., 2010
Wistar rats (F)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	d.w.	700 ppm	21 d	N/A		Soudani et al., 2012
Wistar rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	15 mg/kg	24 h	N/A		García-Niño et al., 2015
Albino Wistar rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	15 mg/kg	24 h	5.4	AAS	Salama et al., 2016
		i.n.	0.5, 1, 2 mg/kg	24 h	0.13, 0.41, 0.92		
Rockfish, <i>S. schlegelii</i>	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Diet	30, 60, 120, 240 mg/kg	2 or 4 weeks	N/A		Kim and Kang, 2016
Guinea pig (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	60 µg/kg	3 weeks	N/A		Fahmy, 2017
Wistar rat (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	2 mg/kg	30 d	N/A		Mahmoud and El-Twab, 2017
Wistar rat (M)	Cr <sub>2</sub> O <sub>3</sub> NP	Oral w/ cannula	0.5 or 2 mg/kg	24 h, 7 d, 14 d	N/A		Fatma et al., 2017
Wistar rat (M)	Cr(OH) <sub>3</sub> NP	Intratracheal instillation	2 mg/kg welding fumes w/ Mn and Fe	once a day, 5x/week, for 4 weeks	N/A		Matié et al., 2017

Species (Gender)	Cr Compound	Exposure Route	Dose(s)	Duration	Cr Level Reported (ug/g)	Method of Detection	Reference
Hyland brown chicken (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	d.w.	(6% LD <sub>50</sub> )	14, 28, 42 d	N/A		Hao et al., 2017
Swiss mice, nulliparous (F)	Tannery effluent	Environmental sludge	500 mL (containing 859 mg/L Cr)	2 h/d, 20 d	N/A		Estrela et al., 2017
Sprague-Dawley rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Oral	25 mg/kg	6 weeks	N/A		Abu Zeid et al., 2018
Zebrafish ( <i>Danio rerio</i> )	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Water	3, 271, 2700 µg/g/L	24 h	N/A		Heffern et al., 2018
<i>Drosophila melanogaster</i>	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Diet	5, 10, or 20 µg/g/mL	24 h or 48h	N/A		Singh and Chowdhuri, 2018
Wistar rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.p.	14 mg/kg (0.5 LD50)	24 h	N/A		Iztleuov et al., 2018
Chicken	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Oral	22.14 mg/kg	14, 28, 42 d	0.1669 ± 0.0041, 0.2520 ± 0.0075, 0.2918 ± 0.0040	ICP-MS	Zhu et al., 2018
Sprague-Dawley rats (F)	K <sub>2</sub> CrO <sub>4</sub>	Oral gavage	10 mg/kg	2 weeks	N/A		Sarica et al., 2019
Zebrafish ( <i>Danio rerio</i> )	K <sub>2</sub> CrO <sub>4</sub>	Water	2 mg Cr/L	1, 7, 15, 30, or 60 d	N/A		Shaw et al., 2020
Japanese Quail ( <i>Coturnix japonica</i> )	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	d.w.	1.2, 2.4 µg/mL	20 d	10.58 ± 4.5 10.62 ± 6.94	USEPA Methods 3060A and 7196A: alkaline digestion and colorimetric spectrophotometry	Suljevi et al., 2021
Wistar rats (M)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	i.n.	0.125, 0.25, 0.5 mg/kg/d	5 d per week, 2 weeks and 2 months	N/A		Hegazy et al., 2021

NP = nanoparticles; i.p. = intra peritoneal; d.w. = drinking water; i.p. = intra peritoneal; d.w. = drinking water; i.n. = intra-nasal AAS = atomic absorption spectrometry; ICP-MS = inductively coupled plasma-mass spectrometry; N/A = not assessed

Table 4.

Summary of Redox Data from Cr (VI) Neurotoxicity Animal Studies

Species (Exposure Route)	Dose, Duration	Lipid Peroxidation	Non-Enzymatic Antioxidant		Enzymatic Antioxidant Activity			Other Oxidative Stress or Damage Measure	Reference
			α-tocopherol	Vit E and C	SOD	CAT	GPx/GR		
Mouse (oral)	1.9, 19, 95 mg/kg, 12, 24, 48, 72, 96 h	I <sup>-</sup> MDA: 1.9 = unaffected; 19 = 1.6x incr after 72h; 95 = 3.2x incr after 48h	N/A		N/A	N/A	N/A	N/A	Bagchi et al., 2002
Mouse (d.w.)	25 mg/kg, 3 d	TBARS incr 29%	α-tocopherol decr 32%	Vit E and C unaffected	Incr 65%	Incr 74%	GPx unaffected		Travacio et al., 2000
Mouse (oral)	25 mg/kg, 6 wk	MDA incr 1.92x	GSH decr 58%		N/A	N/A	GR decr 55%	Protein carbonyl incr 2x	Abu Zeid, 2018
Rat (d.w.)	100 ppm (11.6 mg Cr/kg/d), 30 d	MDA incr: HT = 30% AP = 65%	N/A		Incr 2x in AP	Unaffected	GR incr 2x in HT; GPx unaffected	N/A	Nudler et al., 2009
Rat (d.w.)	700 ppm, 21 d	MDA incr: CTX = 1.2x CB = 94%	GSH decr: CTX = 52% CB = 15%	NPSH decr: CTX = 37% CB = 46%	N/A	N/A		N/A	Soudani et al., 2012
Rat (oral)	0.5 or 2 mg/kg, 24 h, 7 d, 14 d	MDA incr: 24 h = unaffected 7 d (2 mg/kg) = 4x 14 d = 6.7x, 9.3x	GSH decr: 24 h = 40%, 70% 7 d = 53%, 76% 14 d = 88%, 95%		SOD: 24 h = Unaffected, 1.8x incr 7 d = unaffected, 33% decr 14 d = 50%, 75% decr	N/A	N/A	N/A	Fatima et al., 2017
Rat (i.p.)	2 mg/kg, 30 d	MDA incr 2.5x (CTX)	GSH decr 30%		Decr 50%	N/A	GPx decr 40%	NO incr 1.8x (CTX)	Mahmoud and El-Twab, 2017
Rat (oral)	10 mg/kg, 2 wk	MDA incr 1.9x	GSH unaffected		Nonsig decr 23%	Nonsig decr 42%	N/A	N/A	Sarica et al., 2019
Rat (i.p.)	15 mg/kg, 24 h	Plasma MDA incr 88%	N/A		Unaffected	Unaffected	Both unaffected; GPx non-significant 33% decr	N/A	García-Niño et al., 2015

Species (Exposure Route)	Dose, Duration	Lipid Peroxidation	Non-Enzymatic Antioxidant		Enzymatic Antioxidant Activity			Other Oxidative Stress or Damage Measure	Reference
			GSH decr: 1 = 88% 2 = 75%	Non-protein thiols decr 36%	SOD	CAT	GPx/GR		
Rat (i.n.)	0.5, 1.0, 2.0 mg/kg, 24 h	MDA incr: 0.5 = 1.21x 1.0 = 1.61x 2.0 = 2.21x	GSH decr: 1 = 88% 2 = 75%		N/A	Decr: 1.0 = 72% 2.0 = 30%	N/A	N/A	Salama et al., 2016
Rat (i.p.)	15 mg/kg, 24 h	MDA +67%	GSH decr 42%	Non-protein thiols decr 36%	Unaffected	Decr 33%	GR decr 25%		Izileuov et al., 2018
Zebrafish (water)	2 mg/L, 1, 7, 15, 30, 60d	MDA incr 1.77x on day 15	GSH incr 1.33x on day 60		N/A	15d = 1.58x 30d = 2.58x 60d = 2.35x	N/A	N/A	Shaw et al., 2020
Rat (i.n.)	0.125, 0.25, 0.5 mg/kg/d, 2 weeks	MDA incr: 0.125 = 1.23x 0.250 = 1.77x 0.500 = 2.65x	GSH decr: 0.125 = 18% 0.250 = 40% 0.500 = 42%		N/A	N/A	N/A	N/A	Hegazy et al., 2021
Rat (i.n.)	0.125, 0.25, 0.5 mg/kg/d, 1 month	MDA incr: 0.125 = 4.34x 0.250 = 7.70x 0.500 = 7.98x	MDA incr: 0.125 = 45% 0.250 = 52% 0.500 = 52%		N/A	N/A	N/A	N/A	
Rat (i.n.)	0.125, 0.25, 0.5 mg/kg/d, 2 months	MDA incr: 0.125 = 7.31x 0.250 = 8.00x 0.500 = 8.21x	MDA incr: 0.125 = 54% 0.250 = 55% 0.500 = 59%		N/A	N/A	N/A	N/A	

*Nota Bene:* Data was adjusted to percent of control based on reported mean values.

N/A = not assessed; CTX = cortex/cerebrum; CB = cerebellum; HT = hypothalamus; AP = anterior pituitary; i.p. = intra peritoneal; d.w. = drinking water

<sup>1</sup>Data reflect largest observed effect

Table 5.

## Summary of Cr (VI) Cell Culture Neurotoxicology Studies

Cell Line	Cell Type	Compound	Dose(s)	Duration	Observations	Reference
Primary anterior pituitary	Primary, rat	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	0, 0.1, 1, 10 uM	48 h, 72 h	Lactotrophs were more sensitive than gonadotrophs; 10 uM Cr induced ROS for first 2 h; caspase-3 activated after 10 uM Cr for 6, 12, 24 h	Quinteros et al., 2007
Primary anterior pituitary	Primary, rat	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	10 uM	Hours, up to 24 h	Increased PFTα suggests p53 is not involved in Cr(VI)-induce apoptosis; oxidative stress-mediated apoptosis	Quinteros et al., 2008
Cerebellar granule cells	Primary, mouse	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	0.1 – 100 uM	24 h, 48 h	Mature neurons are more sensitive to Cr(VI) toxicity than immature neurons	Dashti et al., 2014
PC12	Rat pheochromocytoma	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	1 – 100 uM	24 h, 48 h, 72 h	ROS and lipid peroxidation significantly increased	Dashti et al., 2015
SH-SY5Y	Human neuroblastoma	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	1 – 10 uM	24 h	Cr(VI) induces neuronal toxicity through ROS-mediated mitochondrial-dependent apoptosis	Fu et al., 2020