

Oral Chromium Exposure and Toxicity

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Abstract Hexavalent chromium [Cr(VI)] is a known carcinogen when inhaled. However, inhalational exposure to Cr(VI) affects only a small portion of the population, mainly by occupational exposures. In contrast, oral exposure to Cr(VI) is widespread and affects many people throughout the globe. In 2008, the National Toxicology Program (NTP) released a 2-year study demonstrating that ingested Cr(VI) was carcinogenic in rats and mice. The effects of Cr(VI) oral exposure are mitigated by reduction in the gut; however, a portion evades the reductive detoxification and reaches target tissues. Once Cr(VI) enters the cell, it ultimately gets reduced to Cr(III), which mediates its toxicity via induction of oxidative stress during the reduction while Cr intermediates react with protein and DNA. Cr(III) can form adducts with DNA that may lead to mutations. This review will discuss the potential adverse effects of oral exposure to Cr(VI) by presenting up-to-date human and animal studies, examining the underlying mechanisms that mediate Cr(VI) toxicity, as well as highlighting opportunities for future research.

Keywords Hexavalent chromium · Oral exposure · Oxidative stress · Reduction · Carcinogenesis

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Introduction

Hexavalent chromium, Cr(VI), is widely used in numerous industrial processes, including chrome pigment production, chrome plating, stainless steel manufacturing, and leather tanning [1, 2]. Numerous epidemiological studies have reported a high incidence of lung cancer among workers exposed occupationally to Cr(VI) by inhalation [3, 4]. The International Agency for Research on Cancer (IARC) has classified Cr(VI) as a human carcinogen through the inhalation route of exposure [5]. However, there are limited human studies on the health effects of Cr(VI) when ingested. Chromate is a very common contaminant in drinking water. In the past, there was extensive discharge to the environment and uptake via aquifers of chrome plating baths and water containing chromate as an antirust agent. Individuals with wells are subject to chromate contamination in their wells via aquifers. A study in China reported an increase of stomach cancer mortality in the area with elevated Cr(VI) concentrations in drinking water, but in this study, there were some limitations with its statistical power [6]. A meta-analysis of data on chromate workers did not support the association between occupational Cr(VI) exposure by inhalation and tumors in the digestive system [7–9]. In 2008, The National Toxicology Program (NTP) released a 2-year rodent study on oral exposure of Cr(VI) in drinking water, indicating that Cr(VI) was a carcinogen when ingested [10, 11]. Since then, a series of studies have been conducted to investigate the association between oral exposure to Cr(VI) and gastrointestinal cancer as well as to establish the modes of action (MOA) for small intestinal tumors that occurred in mice in the NTP study. In this review, we will focus on the recent progress regarding the toxicity and carcinogenicity of oral exposure to Cr(VI).

Chemical Properties

Chromium is one of the most abundant elements in the Earth's crust, with an average concentration of 125 mg/kg [2]. Chromium has multiple oxidation states, ranging from Cr(0) (elemental chromium) to Cr(VI) (hexavalent chromium). The most common as well as stable form of chromium is the trivalent form, Cr(III). Cr(III) occurs naturally in chromite ore and is normally used for manufacturing chromium metal and mono- or di-chromates [2]. The next most stable form is hexavalent chromium. This form occurs rarely in nature and is mainly produced from industrial activities [12]. Cr(VI) and Cr(III) differ not only in their oxidation states but also in their chemical properties and toxicity. While Cr(III) serves as a nutritional supplement, and may play a role in glucose and lipid metabolism, Cr(VI) is very toxic inducing a wide variety of injuries in cells such as DNA damage, chromosomal aberrations, alterations in the epigenome, and microsatellite instability [13–15].

Another difference between Cr(VI) and Cr(III) is their ability to enter cells, which is the basis of the almost 1000-fold difference in their toxicities. Cr(VI) is structurally similar to sulfate and phosphate anions; therefore, cells readily take it up via non-specific anion transporters [13]. Once inside the cell, Cr(VI) undergoes a rapid metabolic reduction and is converted ultimately to Cr(III) [13]. In contrast, Cr(III) compounds cannot enter cells by any transport mechanism [16, 17].

Source of Oral Exposure

The primary source of oral exposure to Cr for non-occupational human populations comes from food and drinking water. Cr levels in the food range from <10 to 1300 µg/kg, with the highest amount in meat, fish, fruits, and vegetables [18]. The concentration of Cr in uncontaminated water is very low, about 1–10 µg/L in rivers and lakes and 0.2–1 µg/L in rainwater, with an average concentration of 0.3 µg/L in ocean water [2, 19].

Increased industrial applications, however, lead to a large amount of Cr released into soil, ground water, and air. In 2009, the estimated releases of Cr compounds to surface water from domestic manufacturing and processing facilities were 486, 063 lbs [19]. The contamination of Cr(VI) in drinking water was first made known to the public in the Erin Brockovich (Film in 2000), depicting a southern California town of Hinkley. The elevated level of Cr(VI) in drinking water (usually several oob) has been reported in more than 30 US cities [20], posing an important question as to the health effect of Cr(VI) exposure in drinking water. The current drinking water standard established by the US Environmental Protection Agency (EPA) for total chromium is 0.1 mg/L or 100 ppb [21]. However, there is no specific drinking water standard

for hexavalent chromium. The California public health goal for Cr(VI) is 0.02 ppb which is a very low level and is often exceeded in public drinking water.

Chromium Metabolism and Reduction

After Cr(VI) enters the cell via anion transporters, it undergoes a series of metabolic reductions and forms intermediate Cr species, Cr(V) Cr(IV), and is finally reduced to Cr(III) [12, 13]. At physiological pH, intracellular reduction of Cr(VI) is facilitated by a number of non-enzymatic and enzymatic antioxidants. Ascorbate (Asc), reduced glutathione (GSH), and cysteine (Cys) are three non-enzymatic reducing agents for Cr(VI) [12, 14]. The primary reducing agent depends on the cellular availability as well as reaction rate. Although Asc and GSH have a similar concentration in vivo (~1–3 mM), early studies showed that Asc was the kinetically favored reducing agent and accounted for 80–90 % of in vivo Cr(VI) reduction [22–24]. At a concentration of 1 mM in vitro, Asc-mediated reduction was more rapid ($T_{1/2}=1$ min), as compared to 60.7 min for GSH and 13.3 min for Cys [25]. However, the amount of Asc in vitro (<50 µM in culture medium) is much less compared to its concentration in vivo (~1–3 mM). Therefore, the reduction of Cr(VI) in cultured cells is primarily facilitated by GSH [12].

Depending on the nature of the reducing agents in the cell, Cr(VI) undergoes either one- or two-electron reductions [13, 14, 26]. Asc reduces Cr(VI) via a two-electron reaction forming the reduction intermediate, Cr(IV). Reduction of Cr(VI) by GSH can be either by one- or two-electron reactions which produce Cr(V) or Cr(IV). Reduction by Cys is almost exclusively a one-electron reaction. A combined activity of Asc, GSH, and Cys in cells reduced more than 95 % of Cr(VI) into Cr(III) [12]. Other intracellular reducing agents include cytochrome P450 reductase, mitochondrial electron transport complexes, glutathione reductase, aldehyde oxidase, etc. Mitochondrial electron transport complexes are potent Cr(VI) reducing agents; however, they only reduce Cr(VI) in the mitochondria. P450 reductase reduces Cr(VI) only in the absence of oxygen. These enzymes are only minor players in the intracellular Cr(VI) reduction [14].

Cr(VI) reduction occurs both inside and outside the cell. Due to its weak membrane permeability, the final metabolite Cr(III) is normally retained in the same place it was produced. For example, intracellular Cr(VI) reduction leads to a massive intracellular accumulation of Cr(III) ranging from 10- to 20-fold after 3 h up to about 100-fold after 24 h of exposure [27, 28]. High levels of Cr(III) in cells react with DNA, which is the principle mechanism underlying Cr(VI) genotoxicity [13–15]. In contrast, Cr(III) generated from extracellular reduction cannot enter the cell and poses little or no toxic and

carcinogenic activity, rendering the extracellular reduction process as a detoxification mechanism.

The major extracellular reduction of Cr(VI) occurs in the gastrointestinal system. After ingestion, Cr(VI) can be reduced to Cr(III) by bodily fluids including saliva and gastric juice and further sequestered by intestinal bacteria [29, 30]. The main reducing agent, gastric juice, has a relatively high reducing capacity, ranging from ~8 mg/L (fasting) to 31 mg/L (Fed). Considering the large amount of fluids secreted in the stomach daily, about 1000–1500 ml/day (fasting) plus 800 ml/meal (Fed), the body has a large capacity to convert Cr(VI) to Cr(III) (~80 mg/day) [29]. Thus, extracellular Cr(VI) reduction, primarily in the stomach, has been considered a protective mechanism accounting for the low genotoxicity and carcinogenicity in animals exposed to Cr(VI) via drinking water [30, 31]. However, recent animal studies by NTP and others demonstrated the systemic intracellular presence of Cr in many tissues and organs, indicating that a portion of Cr(VI) is able to escape detoxification by the gastrointestinal system [10, 32–34, 35•].

Toxicity and Carcinogenicity of Cr(VI) in Drinking Water

Epidemiological Studies

Until now, there have been only a few human studies addressing oral exposure to chromium and its adverse health effects. A study in China reported an increase of stomach cancer mortality in the residents of small villages in the Liaoning province of China where the drinking water was heavily contaminated with Cr(VI) (>0.5 mg/l) [6]. The same investigator published another paper where they reported no risk of cancer mortality; this was retracted in 2006 due to the failure to disclose financial support from industry. A re-analysis of the same data confirmed the increased incidence of stomach cancer in the exposed villages compared to the unexposed, control population in the whole province [36]. However, analyzing the same data using a smaller number of controls from nearby areas with no Cr(VI) in ground water, the association between Cr(VI) exposure and cancer mortality was not replicated [37].

Several areas of Greece have also suffered the consequences of drinking Cr(VI)-contaminated water. An ecological mortality study in the Oinofita region of Greece, where water was contaminated with Cr(VI) (maximum levels ranging between 41 and 156 µg/L), indicated that there was a significantly increased incidence of liver cancer mortality ($p < 0.001$) as well as lung cancer ($p = 0.047$), cancers of the kidney, and other genitourinary organs among women ($p = 0.025$) [38]. A more recent study from Greece reported an

association between chromium exposure in drinking water and some hematological and biochemical parameters [39].

A study in India evaluated adverse health effects on a population exposed to high concentrations of Cr(VI) (~20 mg/L) [40] and reported a slightly increased incidence of gastrointestinal and dermatological complaints in an area with Cr(VI)-contaminated ground water. Although data from human studies are quite limited, the results from all four studies suggest that there were adverse health effects from oral exposure to Cr(VI).

Animal Studies

There were a number of studies conducted in rodents to examine various genotoxic endpoints in target organs. These studies involve short-term Cr(VI) exposure, and no tumor formation would have been observed given the short exposure interval (summarized and discussed in two review articles [41, 42]). The following discussion focuses on the studies associated with carcinogenic endpoints (Table 1).

In 1968, a study reported stomach cancer in mice orally exposed to Cr(VI) in drinking water [43]. In this study, 120 female and 10 male NMRI mice were exposed to 500 ppm of potassium chromate in drinking water for their lifetime. Exposed mice were mated during the exposure. The treatment time was about 880 days and covered three generations. At the time of termination, 2 carcinomas and 9 benign tumors were observed in the forestomach among 66 mice over the combined 3 generations exposed to Cr(VI) [43]. This was statistically significant in comparison to 2 benign tumors in 79 control mice. This result indicated an increased incidence of stomach tumor in Cr(VI)-exposed mice. However, an unexpected outbreak of mousepox virus resulted in high mortality rates of parental mice, and a subsequent mousepox vaccination given to the mice may have compromised the findings.

In 2008, NTP initiated a 2-year rodent study to examine the possible effect of chronic oral exposure to Cr(VI) in their drinking water [10]. The groups of 50 males and 50 females of F344/N rats and B6C3F1 mice were exposed to 0, 14, 57, 172, and 516 mg/L sodium dichromate dehydrate (SDD) [equivalent to 0, 5, 20, 60, and 180 mg/L Cr(VI)] in drinking water. The study was completed after 2 years, and multiple endpoints including both neoplastic and non-neoplastic lesions were assessed. There was clear evidence of carcinogenic activity of Cr(VI) in both rats and mice. Both female and male F344/N rats that were exposed to the highest concentration of Cr(VI) in drinking water developed cancer in the oral cavity. B6C3F1 mice of both sexes, that were exposed to the two highest doses of Cr(VI), developed tumors in the small intestine (duodenum and jejunum) [10, 11]. In addition to tumor formation, increased incidence of histiocytic cellular infiltration in several tissues was observed in both rats and mice. An exposure-related microcytic hypochromic anemia occurred in

Table 1 Summary of in vivo carcinogenic studies of hexavalent chromium by drinking water

| Study | Species | Route | Cr(VI) | Dose | Duration | Endpoints |
|------------------------|------------------------|----------------|-----------------------------|---|---|--|
| Borneff et al. [43] | NMR1 mice | Drinking water | Potassium chromate | 500 ppm | A total of 880 days for 3 generations (F2 exposed about 510 days) | Combined stomach tumors |
| NTP [10, 11] | F344 rat | Drinking water | Sodium dichromate dehydrate | 0, 14.3, 57.3, 172, or 516 mg/L Equivalent to 0, 5, 20, 60, and 180 mg/L Cr(VI) | 2 years | Mucosa in oral cavity Increased incidence of histiocytic infiltration in multiple tissues Exposure-related anemia |
| | B6C3F1 mice | Drinking water | Sodium dichromate dehydrate | 0, 14.3, 57.3, 172, or 516 mg/L Equivalent to 0, 5, 20, 60, and 180 mg/L Cr(VI) | 2 years | Adenoma and carcinoma in the small intestine Increased incidence of histiocytic infiltration in multiple tissues Diffused epithelial hyperplasia in duodenum and jejunum |
| Davidson et al. [32] | SK1-hrBR hairless mice | Drinking water | Potassium chromate | 0, 0.5, 2.5, and 5 ppm, with or without UVR (1.1 kJ/m ²) | A total of 7 months covering 4 months of Cr(VI) exposure and 6 months UVR | No tumor in Cr(VI) alone A Cr(VI) dose-dependent increase of skin tumors in mice exposed to both Cr(VI) and UVR |
| Wang et al. [44] | C57BL/6 mice | Drinking water | Sodium dichromate dehydrate | 167 mg/L alone or with 58 mg/L sodium arsenite in AOM/DSS pretreated mice | 20 weeks | Increased incidence and multiplicity of colon tumor in AOM/DSS pretreated mice Increased 8-oxo-dG in metal-treated mice |
| 90-day study [45*, 46] | F344 rat | Drinking water | Sodium dichromate dehydrate | 0.3, 4, 14, 60, 170, and 520 mg/L Equivalent to 0.1, 1.4, 4.9, 20.9, 59.3, and 181 mg/L Cr(VI) | 7 and 90 days | Significant dose-dependent decrease of GSH/GSSH ratio in the oral mucosa Histiocytic infiltration in the duodenum |
| | B6C3F1 mice | Drinking water | Sodium dichromate dehydrate | 0.3, 4, 14, 60, 170, and 520 mg/L Equivalent to 0.1, 1.4, 4.9, 20.9, 59.3, and 181 mg/L Cr(VI) | 7 and 90 days | Significant dose-dependent decrease of GSH/GSSH ratio in the duodenum Intestinal lesions Histiocytic infiltration in the duodenum Crypt cell hyperplasia |

rats, but only a mild erythrocyte microcytosis was seen in mice. Diffused epithelial hyperplasia was only observed in the duodenum and jejunum of mice but not rats. These data, which are consistent with many *in vitro* studies, further supported the notion that Cr(VI) is a carcinogen when ingested. A more detailed discussion on the major finding and conclusions from the NTP study was provided in a recent review article [35•].

Prior to the NTP study, Davidson et al. reported a study using a hairless mouse model to assess the toxic and carcinogenic effect of Cr(VI) in drinking water [32, 33]. The hairless mice were treated with low doses of ultraviolet radiation in the absence and presence of various doses of potassium dichromate (0.5, 2.5, and 5 ppm) in drinking water for 6 months. While ingestion of Cr(VI) alone in drinking water did not produce any skin tumors, co-treatment with a low dose of UVR displayed a synergistic effect on skin tumor formation [32]. A dose-related increase of tumor numbers was observed with the increased concentration of Cr(VI) in drinking water. The results indicated that, while Cr(VI) alone was not sufficient to induce tumor formation in the skin in a relative short exposure duration and at low doses that have occurred in human exposure scenarios, Cr(VI) in drinking water can promote UV-induced tumorigenesis at a site that was distant from its entry point (ingestion). Most importantly, this study demonstrated the ability of ingested Cr(VI) to evade reduction in the stomach and distribute to multiple tissues including the skin, liver, kidney, and bone [1, 32, 33]. Later, another study using a mouse colitis-associated colorectal cancer model showed that exposure to Cr(VI) and arsenic in drinking water for 20 weeks, alone or in combination, promoted azoxymethane/dextran sodium sulfate (AOM/DSS)-induced tumorigenesis. The authors further showed that tumor promotion activity of Cr(VI) and arsenic was mediated through ROS-induced Wnt/beta-catenin signaling pathway [47].

Mechanisms of Chromium Toxicity and Carcinogenicity

Oxidative Stress Cr(VI) is able to induce oxidative stress via multiple pathways [26]. First, the metabolic intermediates and ultimate products generated during Cr(VI) reduction can participate in Fenton-type reactions to generate hydroxyl radicals in the presence of hydrogen peroxide. Alternatively, in the presence of endogenous superoxide anion and hydrogen peroxide, Cr(V) and Cr(VI) can produce hydroxyl radicals via Haber-Weiss-like reactions. Moreover, by forming Cr-Asc, Cr-GSH, and Cr-cys crosslinks, reduction of Cr(VI) depletes cellular antioxidants and disrupts the redox balance in the cell [26].

Depending on the levels of ROS production, Cr(VI)-induced oxidative stress may lead to cell death (cytotoxicity) or tumor formation (carcinogenicity). High levels of ROS production directly target lipid and DNA to generate lipid peroxidation and DNA damage as well as many other cellular injuries, leading to cell death by both apoptosis and necrosis. Medium to low levels of ROS in cells may disrupt the cellular redox balance and accelerate cell proliferation, leading to tumor formation and progression. 8-oxo-dG, a marker for oxidative DNA damage, has been detected *in vitro* in Cr(VI) exposed cells in many studies. However, the levels of 8-oxo-dG were not induced in the intestine of mice following Cr(VI) exposure in drinking water for 9 months [44] or 3 months [45•]. It is possible that a majority of ingested Cr(VI) was reduced to Cr(III) by gastric juice, and the small amount of Cr(VI) absorbed by cells could not induce detectable 8-oxo-dG. It is worth noting that 8-oxo-dG has a relative short lifetime in cells [12], and there is high background when measuring 8-oxo-dG suggesting some technical problems in assessing this DNA adduct.

Recently, Thompson et al. reported a dose- and time-dependent decrease in the reduced-to-oxidized glutathione ratio (GSH/GSSG) in the duodenum of B6C3F1 mice exposed to various doses of Cr(VI) in drinking water for 7 or 90 days [45•], indicating that chronic low-dose exposure of Cr(VI) in drinking water can induce oxidative stress in target tissues. Subsequent gene expression analysis revealed that 3 out of 16 genes that satisfy the dose-dependent differential expression criteria with EC(50) <10 mg/L SDD are Nrf2 downstream genes [48]. Nrf2 is a transcription factor that can be activated by ROS. Genes downstream of Nrf2 are normally involved in oxidative stress responses. There is clear evidence of villous cytotoxicity and focal or diffused hyperplasia in the small intestine in both the NTP study and the 90-day animal study [10, 45•], which is likely the consequence of increased oxidative stress. It is possible that low doses of Cr(VI) exposure via drinking water induced chronically elevated levels of ROS, which subsequently contributed to initiate tumor formation by inducing mutations in DNA, increasing cell proliferation, altering the epigenome, inhibiting cell apoptosis, and promoting tumor progression.

DNA Damage and Cr(VI) Mutagenicity

Cr(VI) itself does not bind to DNA or other macromolecules in cells. Instead, its metabolic intermediates Cr(V) and Cr(IV) and the final product Cr(III) are highly reactive and readily form Cr-DNA adducts. Cr(III)-induced DNA adducts can be either binary (Cr-DNA) or ternary (ligand-Cr-DNA), where the ligand can be Asc, GSH, cysteine, histidine, or other cysteine-containing proteins [13]. Binary Cr-DNA adducts can be repaired rather efficiently, within minutes after exposure by nucleotide excision repair (NER) in cells. NER-

deficient human cells are hypersensitive to Cr(VI) toxicity with a massive accumulation of Cr-DNA adducts [49], suggesting that DNA repair plays an important role in antagonizing Cr(VI)-induced DNA damage. Ternary Cr-DNA adducts are strong inhibitors of DNA replication and transcription. Interestingly, replication inhibition of ternary Cr-DNA adducts requires the presence of mismatch repair (MMR) proteins [50]. MMR-null mice and human cells were resistant to cytotoxicity induced by Cr(VI) [50–52].

In addition to binary and ternary Cr-DNA adducts, Cr(VI) reduction can generate several other DNA or chromosome lesions, including abasic sites, single- and double- strand breaks, protein-Cr-DNA crosslinks, DNA inter/intrastrand crosslinks, etc [12, 14, 15]. DNA single- or double- strand breaks have been detected in animals that were orally exposed to Cr(VI) by gavage [41, 42]. DNA-protein crosslinks were detected in liver cells but not lymphocytes from F344 rats that were exposed to potassium chromate via drinking water for 3 weeks [53]. However, DNA-protein crosslink was not induced in mice exposed to Cr(VI) for 9 months via drinking water [44]. The ability of these DNA lesions to induce DNA mutations has been extensively studied in vitro using the shuttle-vector system [12, 14] and in vivo using the Big Blue Mouse model [54]. P53 point mutations have been reported at higher frequencies in lung tumors from chromate workers than lung samples from non-chromate workers [55]. Very few studies have examined DNA mutation in animals orally exposed to Cr(VI). In one study, K-Ras codon 12 GAT mutations were observed in both Cr(VI)-treated and Cr(VI)-untreated mice, without a clear treatment-related trend [56].

Cr(VI) exposure also induced genomic changes in a larger scale than the DNA damage described above. The microsatellite instability and chromosome aberrations were observed in many Cr(VI)-exposed biological systems, both in vitro and in vivo [4, 57]. More than 15 studies analyzed micronucleus formation as the genotoxic endpoint in animals exposed to Cr(VI) via oral administration at various doses and exposure durations; however, the results from these studies are mixed. In most studies, micronucleus formation was analyzed in normochromatic erythrocytes which are not the target cells for oral Cr(VI) exposure. Recently, a significant increase of aberrant nuclei were reported at villi tips in the mouse exposed to Cr(VI) via drinking water for 90 days, but none was detected in the crypt area of the same mouse [56].

Mode of Action Analysis in 90-Day Study

The results from the NTP 2-year study clearly indicated that Cr(VI) is a carcinogen when administered via the oral route. However, several questions and data gaps remain that require further investigation [58•]. A separate 90-day animal

study was conducted to investigate the mode of action (MOA) underlying tumor formation using the same Cr(VI) concentration from the 2-year NTP bioassay but included two additional lower doses: 0.3 and 4 mg/L SDD (equivalent to 0.1 and 1.4 mg/L Cr(VI) in drinking water) [45•]. Although there was no neoplastic effect observed in this study due to the short duration of exposure, analysis of this study provided insights into potential mechanisms underlying Cr(VI)-induced tumor formation. First, the ratio of reduced-to-oxidized glutathione (GSH/GSSG), which is an indicator of oxidative stress, was decreased in both rats [46] and mice [45•], suggesting Cr(VI) exposure caused oxidative stress in both species and also indicating that a substantial portion of Cr(VI) was not reduced in the GI tract. While total Cr levels were comparable in the rat and mouse, the reduced GSH/GSSG ratio was only observed in rat oral mucosae and mouse duodenum, the sites that formed tumors in the NTP 2-year study [45•, 46]. Subsequent gene expression analysis revealed that 3 out of 16 identified genes are Nrf2 downstream genes [48], which further support Cr(VI) induced oxidative stress in the duodenum of mice. Second, pathological analysis observed a number of intestinal lesions including villous cytoplasmic vacuolization, atrophy, and apoptosis. Crypt hyperplasia was also observed in the small intestine [42, 45•]. Third, a further analysis of micronucleus in the mouse duodenum revealed an increased incidence of aberrant nuclei in the villi, a place of tissue damage, but not in the crypt compartment where cell proliferation occurs [56]. Recent analysis of r-H2AX by immunostaining obtained similar patterns, with elevated levels in villi but not the crypt compartment [59]. Lastly, high spontaneous K-Ras mutations and lack of treatment-related trend in K-Ras mutation frequency were found in the duodenum of mice [56]. Taken together, the results from the 90-day study suggested a non-mutagenic MOA that involves chronic wounding of intestinal villi and crypt cell hyperplasia, both of which might contribute to tumor formation [42, 59]. However, a relative short-term exposure is a shortcoming of this study. The changes associated with tumor formation may not have occurred yet or be detected at the early time period chosen for this study. The results cannot represent or predict the later changes that may have driven tumor formation.

Reduction Capacity of the Gastrointestinal System

The lowest dose of Cr(VI) (60 mg/L) that induced tumor formation in the 2-year NTP study was much higher than the EPA standard for drinking water (0.1 mg/L total chromium standard), which raises a series of questions regarding how the conclusions of this study can be extrapolated to human exposure. Extracellular reduction of Cr(VI) to Cr(III) in the gastrointestinal system has been considered a major detoxification process that attenuates the toxicity and carcinogenicity of

Cr(VI) via oral exposure [29, 30, 60, 61]. It was hypothesized that ingested Cr(VI) may only induce tumors at doses that exceed the reduction capacity of mouse gastrointestinal system, and there is limited risk of ingested Cr(VI) in humans at environmentally relevant doses. However, others argue that the reduction of Cr(VI) to Cr(III) outside of cells and absorption of Cr(VI) by cells via non-specific anion transporters occur almost simultaneously and both processes compete with each other for substrate [12, 62]. This competition combined with other factors including gastric emptying time, food content, and interspecies difference in reduction result in a portion of ingested Cr(VI) (10–20 %) escaping gastric detoxification and being absorbed into cells of target tissues even at very low concentrations [12, 62]. This fact was well supported by the tissue distribution analysis of ingested Cr(VI) in both human and animal studies [32–34]. However, many questions still need to be addressed, including the reduction capacity of gastric fluid, the rates and capacities for Cr(VI) reduction along the GI tract, the rate of Cr(VI) absorption in the GI tract, and the factors that potentially affect Cr(VI) reduction or absorption.

Epigenetic Modulation

In the past decade, a growing body of evidence has suggested that Cr(VI)-induced changes to the epigenetic landscape may contribute to its carcinogenicity [63, 64]. Epigenetic refers to the reversible but inheritable changes in gene expression that occur without alterations in the DNA sequence [65]. DNA methylation, histone modification, and microRNA are major components of epigenetic regulation.

DNA methylation is a covalent modification in which a methyl group is added to the 5'-carbon of cytosine. An investigation by Klein et al. presented the first evidence that Cr(VI) could aberrantly induce DNA methylation and silenced the *gpt* transgene [66]. Lung cancers from chromate workers exposed to Cr(VI) via inhalation displayed increased DNA methylation in the promoter of the tumor suppressor gene, *P16*, leading to *P16* gene silencing [67]. Silencing of the mismatch repair gene, *MLH1*, in lung cancers of the chromate workers was associated with high replication error and microsatellite instability [68]. Ali et al. reported hypermethylation of the *APC*, *MGMT*, and *hMLH1* gene promoters in chromate lung cancers compared with those in non-chromate lung cancer [69]. Recently, our group analyzed the promoter methylation of 22 tumor suppressor genes in workers from a chromate factory and referent subjects using EpiTect Methyl II PCR array. Among 22 tumor suppressor gene promoters whose hypermethylation was frequently observed in many human cancers, three genes (*WIFI*, *APC*, and *MLH1*) exhibited elevated promoter DNA methylation in chromate workers compared to referent subjects [70]. Other than gene-specific changes, Wang et al (2012) reported global hypomethylation

in chromate workers that have not developed any tumor symptoms [71]. A recent in vitro study also reported that global DNA hypomethylation was observed in less than 2 h of Cr(VI) exposure in two human cell lines [72].

In addition to DNA methylation, posttranscriptional modification of histone tails and microRNA expression are also the targets of Cr(VI) exposure. It has been reported that Cr(VI) exposure in vitro resulted in decreased histone acetylation and increased histone biotinylation [73, 74], and increased or decreased histone methylation in both global and gene-specific manner [75, 76]. Moreover, miR-143 was decreased in a chromium-transformed cell line [77], and plasma miR-3940-5p levels were negatively associated with Cr(VI) exposure in chromate workers [78].

Given the capacity of Cr(VI) to modulate epigenetic components, it is possible that epigenetic mechanisms may mediate, at least partly, the carcinogenic effects of oral Cr(VI) exposure. It would be interesting and important to investigate the epigenetic changes in future human or animal studies on Cr(VI) oral exposure.

Conclusion

The carcinogenic potential of oral exposure to Cr(VI) in human is supported by a number of new studies discussed in this review. However, due to extracellular reduction of Cr(VI) to Cr(III) in the gastrointestinal system, oral exposure results in a lower amount of Cr(VI) that gets into target cells compared to inhalation exposure. According to the US EPA, mutagenic carcinogens have no threshold. This may be one reason that industry has sponsored a number of studies that support a non-mutagenic mode of action for Cr(VI), so that the concept of a safe dose can be applied by the regulatory agencies. A portion of ingested Cr(VI) may enter cells by evading extracellular reduction in the stomach. Any amount of Cr(VI) entering cells has the potential to initiate tumor formation. Therefore, Cr levels in drinking water must be set at levels that protect the entire population (young and old) and they should be based on the hexavalent form and not total Cr.

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Compliance with Ethics Guidelines

Conflict of Interest All authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any new studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance

- Costa M, Klein CB. Toxicity and carcinogenicity of chromium compounds in humans. *Crit Rev Toxicol*. 2006;36:155–63.
- Langard S, and Costa M: Chapter 33: Chromium. In: Nordberg GF, Fowler BA, and Nordberg M, editors. *Handbook on the toxicology of metals*. Academic Press; 2014. pp. 717–742.
- Gibb HJ, Lees PS, Pinsky PF, et al. Lung cancer among workers in chromium chemical production. *Am J Ind Med*. 2000;38:115–26.
- Holmes AL, Wise SS, Wise JP. Carcinogenicity of hexavalent chromium. *Indian J Med Res*. 2008;128:353–72.
- IARC. Chromium, nickel and welding. IARC monographs on the evaluation of carcinogenic risks to humans. *Int Agency Res Cancer Lyon*. 1990;49:49–256.
- Zhang JD, Li XL. Chromium pollution of soil and water in Jinzhou. *Zhonghua Yu Fang Yi Xue Za Zhi*. 1987;21:262–4.
- Cole P, Rodu B. Epidemiologic studies of chrome and cancer mortality: a series of meta-analyses. *Regul Toxicol Pharmacol*. 2005;43:225–31.
- Gatto N, Alexander D, Kelsh M. Meta-analysis of occupational exposure to hexavalent chromium and stomach cancer. *Epidemiology*. 2007;18:S33.
- Gatto NM, Kelsh MA, Mai DH, et al. Occupational exposure to hexavalent chromium and cancers of the gastrointestinal tract: a meta-analysis. *Cancer Epidemiol*. 2010;34:388–99.
- National Toxicology Program: Toxicology and carcinogenesis studies of sodium dichromate dihydrate (Cas No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). *Natl Toxicol Program Tech Rep Ser*. 2008; 1-192.
- Stout MD, Herbert RA, Kissling GE, et al. Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. *Environ Health Perspect*. 2009;117:716–22.
- Zhitkovich A. Chromium in drinking water: sources, metabolism, and cancer risks. *Chem Res Toxicol*. 2011;24:1617–29.
- Zhitkovich A. Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium (VI). *Chem Res Toxicol*. 2005;18:3–11.
- O'Brien T. Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms. *Mutat Res/Fundam Mol Mech Mutagen*. 2003;533:3–36.
- Nickens KP, Patierno SR, Ceryak S. Chromium genotoxicity: a double-edged sword. *Chem Biol Interact*. 2010;188:276–88.
- Costa M. Toxicity and carcinogenicity of Cr(VI) in animal models and humans. *Crit Rev Toxicol*. 1997;27:431–42.
- Salnikow K, Zhitkovich A. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem Res Toxicol*. 2008;21:28–44.
- World Health Organization (WHO). Chromium in drinking-water—background document for development of WHO guidelines for drinking-water quality, WHO, 2003; Geneva Switzerland.
- Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for chromium, U.S. Department of Health and Human Services, 2012; Washington, DC.
- Sutton R, Group EW. Chromium-6 in US tap water, (Environmental Working Group). 2010.
- Environmental Protection Agency (EPA). National primary drinking water regulations; final rule. *Fed Regist*. 1991;56:3536–7.
- Suzuki Y, Fukuda K. Reduction of hexavalent chromium by ascorbic acid and glutathione with special reference to the rat lung. *Arch Toxicol*. 1990;64:169–76.
- Standeven AM, Wetterhahn KE. Ascorbate is the principal reductant of chromium (VI) in rat liver and kidney ultrafiltrates. *Carcinogenesis*. 1991;12:1733–7.
- Standeven AM, Wetterhahn KE. Ascorbate is the principal reductant of chromium(VI) in rat lung ultrafiltrates and cytosols, and mediates chromium-DNA binding in vitro. *Carcinogenesis*. 1992;13:1319–24.
- Queivryn G, Peterson E, Messer J, et al. Genotoxicity and mutagenicity of chromium(VI)/ascorbate-generated DNA adducts in human and bacterial cells. *Biochemistry*. 2003;42:1062–70.
- Yao H, Guo L, Jiang BH, et al. Oxidative stress and chromium(VI) carcinogenesis. *J Environ Pathol Toxicol Oncol*. 2008;27:77–88.
- Messer J, Reynolds M, Stoddard L, et al. Causes of DNA single-strand breaks during reduction of chromate by glutathione in vitro and in cells. *Free Radic Biol Med*. 2006;40:1981–92.
- Reynolds M, Stoddard L, Bernal I, et al. Ascorbate acts as a highly potent inducer of chromate mutagenesis and clastogenesis: linkage to DNA breaks in G2 phase by mismatch repair. *Nucleic Acids Res*. 2007;35:465–76.
- De Flora S, Camoirano A, Bagnasco M, et al. Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity. *Carcinogenesis*. 1997;18:531–7.
- De Flora S. Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *Carcinogenesis*. 2000;21:533–41.
- De Flora S, Iltcheva M, Balansky RM. Oral chromium(VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses. *Mutat Res*. 2006;610:38–47.
- Davidson T, Kluz T, Burns F, et al. Exposure to chromium (VI) in the drinking water increases susceptibility to UV-induced skin tumors in hairless mice. *Toxicol Appl Pharmacol*. 2004;196:431–7.
- Uddin AN, Burns FJ, Rossman TG, et al. Dietary chromium and nickel enhance UV-carcinogenesis in skin of hairless mice. *Toxicol Appl Pharmacol*. 2007;221:329–38.
- Collins BJ, Stout MD, Levine KE, et al. Exposure to hexavalent chromium resulted in significantly higher tissue chromium burden compared with trivalent chromium following similar oral doses to male F344/N rats and female B6C3F1 mice. *Toxicol Sci*. 2010;118:368–79.
- Witt KL, Stout MD, Herbert RA, et al. Mechanistic insights from the NTP studies of chromium. *Toxicol Pathol*. 2013;41:326–42. **This study presented the major results and conclusions from two NTP Cr studies and discussed multiple potential mechanisms involved in Cr-induced genotoxicity and carcinogenicity.**
- Beaumont JJ, Sedman RM, Reynolds SD, et al. Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology*. 2008;19:12–23.
- Kerger BD, Butler WJ, Paustenbach DJ, et al. Cancer mortality in Chinese populations surrounding an alloy plant with chromium smelting operations. *J Toxicol Environ Health A*. 2009;72:329–44.
- Linou A, Petralias A, Christophi CA, et al. Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece—an ecological study. *Environ Health*. 2011;10:50.
- Sazakli E, Villanueva CM, Kogevinas M, et al. Chromium in drinking water: association with biomarkers of exposure and effect. *Int J Environ Res Public Health*. 2014;11:10125–45.
- Sharma P, Bihari V, Agarwal SK, et al. Groundwater contaminated with hexavalent chromium [Cr (VI)]: a health survey and clinical examination of community inhabitants (Kanpur, India). *PLoS One*. 2012;7, e47877.

41. Sedman RM, Beaumont J, McDonald TA, et al. Review of the evidence regarding the carcinogenicity of hexavalent chromium in drinking water. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev.* 2006;24:155–82.
42. Thompson CM, Proctor DM, Suh M, et al. Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans. *Crit Rev Toxicol.* 2013;43:244–74.
43. Borneff J, Engelhardt K, Griem W, et al. Carcinogens in water and soil. XXII. Experiment with 3,4-benzopyrene and potassium chromate in mice drink. *Arch Hyg Bakteriol.* 1968;152:45–53.
44. De Flora S, D'Agostini F, Balansky R, et al. Lack of genotoxic effects in hematopoietic and gastrointestinal cells of mice receiving chromium(VI) with the drinking water. *Mutat Res.* 2008;659:60–7.
45. Thompson CM, Proctor DM, Haws LC, et al. Investigation of the mode of action underlying the tumorigenic response induced in B6C3F1 mice exposed orally to hexavalent chromium. *Toxicol Sci.* 2011;123:58–70. **The study shows a significant decrease in the GSH/GSSG ration in the duodenum from Cr(VI) treated mice, suggesting that oral exposure of Cr(VI) in drinking water can induce oxidative stress.**
46. Thompson CM, Proctor DM, Suh M, et al. Comparison of the effects of hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: implications for carcinogenic modes of action. *Toxicol Sci.* 2012;125:79–90.
47. Wang X, Mandal AK, Saito H, et al. Arsenic and chromium in drinking water promote tumorigenesis in a mouse colitis-associated colorectal cancer model and the potential mechanism is ROS-mediated Wnt/ β -catenin signaling pathway. *Toxicol Appl Pharmacol.* 2012;262:11–21.
48. Kopec AK, Kim S, Forgacs AL, et al. Genome-wide gene expression effects in B6C3F1 mouse intestinal epithelia following 7 and 90 days of exposure to hexavalent chromium in drinking water. *Toxicol Appl Pharmacol.* 2012;259:13–26.
49. Reynolds M, Peterson E, Quievryn G, et al. Human nucleotide excision repair efficiently removes chromium-DNA phosphate adducts and protects cells against chromate toxicity. *J Biol Chem.* 2004;279:30419–24.
50. Reynolds M, Zhitkovich A. Cellular vitamin C increases chromate toxicity via a death program requiring mismatch repair but not p53. *Carcinogenesis.* 2007;28:1613–20.
51. Reynolds MF, Peterson-Roth EC, Bepalov IA, et al. Rapid DNA double-strand breaks resulting from processing of Cr-DNA crosslinks by both MutS dimers. *Cancer Res.* 2009;69:1071–9.
52. Peterson-Roth E, Reynolds M, Quievryn G, et al. Mismatch repair proteins are activators of toxic responses to chromium-DNA damage. *Mol Cell Biol.* 2005;25:3596–607.
53. Coogan TP, Motz J, Snyder CA, et al. Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure of rats to potassium chromate. *Toxicol Appl Pharmacol.* 1991;109:60–72.
54. Cheng L, Sonntag DM, de Boer J, et al. Chromium(VI)-induced mutagenesis in the lungs of big blue transgenic mice. *J Environ Pathol Toxicol Oncol.* 2000;19:239–49.
55. Kondo K, Hino N, Sasa M, et al. Mutations of the p53 gene in human lung cancer from chromate-exposed workers. *Biochem Biophys Res Commun.* 1997;239:95–100.
56. O'Brien TJ, Ding H, Suh M, et al. Assessment of K-Ras mutant frequency and micronucleus incidence in the mouse duodenum following 90-days of exposure to Cr(VI) in drinking water. *Mutat Res.* 2013;754:15–21.
57. Wise SS, Wise JP. Chromium and genomic stability. *Mutat Res.* 2012;733:78–82.
58. Thompson CM, Haws LC, Harris MA, et al. Application of the U.S. EPA mode of action framework for purposes of guiding future research: a case study involving the oral carcinogenicity of hexavalent chromium. *Toxicol Sci.* 2011;119:20–40. **The study analyzed the key events in Cr(VI) MOA underlying mouse intestinal tumors, and pointed out the major data gaps and possible research strategies to establish Cr(VI) MOA.**
59. Thompson CM, Seiter J, Chappell MA, et al. Synchrotron-based imaging of chromium and γ -H2AX immunostaining in the duodenum following repeated exposure to Cr(VI) in drinking water. *Toxicol Sci.* 2015;143:16–25.
60. Proctor DM, Otani JM, Finley BL, et al. Is hexavalent chromium carcinogenic via ingestion? A weight-of-evidence review. *J Toxicol Environ Health A.* 2002;65:701–46.
61. Paustenbach DJ, Finley BL, Mowat FS, et al. Human health risk and exposure assessment of chromium (VI) in tap water. *J Toxicol Environ Health A.* 2003;66:1295–339.
62. Stern AH. A quantitative assessment of the carcinogenicity of hexavalent chromium by the oral route and its relevance to human exposure. *Environ Res.* 2010;110:798–807.
63. Arita A, Costa M. Epigenetics in metal carcinogenesis: nickel, arsenic, chromium and cadmium. *Metallomics.* 2009;1:222–8.
64. Brocato J, Costa M. Basic mechanics of DNA methylation and the unique landscape of the DNA methylome in metal-induced carcinogenesis. *Crit Rev Toxicol.* 2013;43:493–514.
65. Rodríguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. *Nat Med.* 2011;17:330–9.
66. Klein CB, Su L, Bowser D, et al. Chromate-induced epimutations in mammalian cells. *Environ Health Perspect.* 2002;110 Suppl 5: 739–43.
67. Kondo K, Takahashi Y, Hirose Y, et al. The reduced expression and aberrant methylation of p16(INK4a) in chromate workers with lung cancer. *Lung Cancer.* 2006;53:295–302.
68. Takahashi Y, Kondo K, Hirose T, et al. Microsatellite instability and protein expression of the DNA mismatch repair gene, hMLH1, of lung cancer in chromate-exposed workers. *Mol Carcinog.* 2005;42: 150–8.
69. Ali AH, Kondo K, Namura T, et al. Aberrant DNA methylation of some tumor suppressor genes in lung cancers from workers with chromate exposure. *Mol Carcinog.* 2011;50:89–99.
70. Sun H, Kluz T, Qu Q, et al.: DNA hypermethylation of tumor suppressor genes in chromate workers (abstract #969). Society of Toxicology. 2014; Phoenix, AZ.
71. Wang TC, Song YS, Wang H, et al. Oxidative DNA damage and global DNA hypomethylation are related to folate deficiency in chromate manufacturing workers. *J Hazard Mater.* 2012;213–214: 440–6.
72. Lou J, Wang Y, Yao C, et al. Role of DNA methylation in cell cycle arrest induced by Cr (VI) in two cell lines. *PLoS One.* 2013;8, e71031.
73. Xia B, Yang LQ, Huang HY, et al. Chromium(VI) causes down regulation of biotinidase in human bronchial epithelial cells by modifications of histone acetylation. *Toxicol Lett.* 2011;205:140–5.
74. Xia B, Ren XH, Zhuang ZX, et al. Effect of hexavalent chromium on histone biotinylation in human bronchial epithelial cells. *Toxicol Lett.* 2014;228:241–7.
75. Sun H, Zhou X, Chen H, et al. Modulation of histone methylation and MLH1 gene silencing by hexavalent chromium. *Toxicol Appl Pharmacol.* 2009;237:258–66.
76. Zhou X, Li Q, Arita A, et al. Effects of nickel, chromate, and arsenite on histone 3 lysine methylation. *Toxicol Appl Pharmacol.* 2009;236:78–84.
77. He J, Qian X, Carpenter R, et al. Repression of miR-143 mediates Cr (VI)-induced tumor angiogenesis via IGF-1R/IRS1/ERK/IL-8 pathway. *Toxicol Sci.* 2013;134:26–38.
78. Li Y, Li P, Yu S, et al. miR-3940-5p associated with genetic damage in workers exposed to hexavalent chromium. *Toxicol Lett.* 2014;229:319–26.