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Review of the Evidence Regarding the Carcinogenicity of Hexavalent Chromium in Drinking Water

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Recent analyses have revealed that 38% of municipal sources of drinking water in California have detectable levels of hexavalent chromium. This observation provided new impetus to characterize the carcinogenic risk associated with oral exposure to hexavalent chromium in drinking water. Notwithstanding the well-characterized increases in cancer associated with inhalation exposure to this chemical, the marked reduction of hexavalent chromium to trivalent chromium in the stomach suggests that exposure to hexavalent chromium in drinking water may not pose a carcinogenic risk. A reevaluation of studies that investigated the toxicokinetics, the genotoxicity, and the mechanism of carcinogenicity of hexavalent chromium, as well as the available human and animal cancer studies, was undertaken to determine if there is evidence that exposure to this chemical in drinking water may pose a carcinogenic risk. Mechanistic studies suggest the potential for a carcinogenic response if hexavalent chromium enters cells. Both toxicokinetic and genotoxicity studies indicate that a portion of an orally administered dose of hexavalent chromium is absorbed and gets into cells of several tissues, causing DNA damage. The only lifetime oral study of hexavalent chromium in animals conducted thus far yielded a statistically significant increase in stomach tumors compared

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Abbreviations. IARC = International Agency for Research on Cancer, NTP = National Toxicology Program, EPA = U.S. Environmental Protection Agency.

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to controls. Also, in a limited-term cancer study, co-exposure to hexavalent chromium in drinking water and ultraviolet light produced skin tumors in mice. The only available cancer study of humans exposed to hexavalent chromium in drinking water revealed a statistically significant increase in stomach tumors. Moreover, a meta-analysis of occupational studies also revealed a statistically significant increase in stomach cancers. The increases in stomach tumors in both human and animal studies, along with the toxicokinetic, genotoxic, and mechanistic data, suggest that oral exposure to this agent appears to pose a carcinogenic risk.

Key Words: Chromium 6+; chromium 3+; Risk Assessment; Drinking Water; Cancer; Hexavalent Chromium; Trivalent Chromium

INTRODUCTION

The extensive use of hexavalent chromium in various industries has resulted in exposure of numerous workers to this chemical, primarily through the inhalation pathway. Several epidemiological studies of these workplaces have yielded sufficient evidence for the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (U.S. EPA) to classify hexavalent chromium as a human carcinogen (1,2), and the State of California to classify hexavalent chromium as a chemical known to the state to cause cancer (3). Recent analyses have revealed detectable levels of hexavalent chromium in 38% of the drinking water sources in California (4). California uses a 1-μg/L detection limit for purposes of reporting. These data suggest that a large population, much larger than chromium workers, may be exposed to hexavalent chromium by the oral route.

In contrast to hexavalent chromium, trivalent chromium is considered to be an essential element, essentially non-toxic, and not posing a significant carcinogenic risk (5). Because oral exposure to hexavalent chromium results in the rapid conversion of hexavalent chromium to trivalent chromium in the acidic environment of the stomach, several investigators have hypothesized that exposure to hexavalent chromium in drinking water does not pose a carcinogenic risk (6–8).

This report evaluates this hypothesis first by reviewing the possible mechanism(s) of hexavalent chromium carcinogenicity. The extensive toxicokinetic and genotoxic literature associated with oral exposures to hexavalent chromium in animals and humans is then discussed. This article also reviews the available cancer studies in experimental animals and humans associated with oral exposure to hexavalent chromium. This effort includes a meta-analysis of occupational studies, undertaken because large inhaled hexavalent chromium-laden particulates would be expect to be cleared from the lung by mucociliary action and then swallowed. A discussion of the consistency and limitations of the totality of available evidence follows.

MECHANISM OF GENOTOXICITY AND CARCINOGENICITY

Although hexavalent chromium has been extensively studied for its genotoxic and carcinogenic potential, the precise mechanism(s) of carcinogenesis is unclear. Hexavalent chromium induces a wide range of DNA damage, including DNA adducts, DNA-protein crosslinks, DNA-DNA crosslinks, mutations, DNA strand breaks, abasic sites, oxidized DNA bases, chromosomal aberrations, sister chromatid exchanges, and micronuclei (9–11). The wide spectrum of genotoxic effects likely reflects multiple mechanisms of DNA damage (11).

Hexavalent chromium appears to be readily taken up by cells because it is a tetrahedral anion (chromate) that mimics phosphate and sulfate salts that are taken up into cells via active transport systems (11). While trivalent chromium appears to be largely excluded from cells because it is not a substrate of the anion transport system, some Cr III probably enters the cell through the energy intensive process of pinocytosis. Cellular uptake of hexavalent and trivalent chromium will be more thoroughly discussed later in this review.

Once taken up by cells, hexavalent chromium is reduced from a +6 oxidation state to trivalent chromium, a +3 oxidation state, in the presence of reducing agents such as ascorbate, glutathione, NADPH and NADH (12–16). While glutathione appears to be the primary reductant of Cr VI in the cytosol, a substantial portion of the reduction of Cr VI is believed to occur in the mitochondrion with NADH as the primary reductant (15–17). The formation of Cr V and Cr IV intermediates from Cr VI have been detected using electron spin resonance spectroscopy techniques in both *in vitro* studies and *in vivo* in animals administered Cr VI (18, 19). It is during the reduction of hexavalent chromium to trivalent chromium that many DNA-reactive species are formed, including free radicals such as hydroxyl radical, singlet oxygen, superoxide anion (O_2^-), glutathione and other thiol radicals, and carbon-based radicals (9–11). Cr V and Cr IV have been shown to react with H_2O_2 to generate hydroxyl radicals through Fenton reactions (20, 21).

Hydroxyl radicals that induce DNA double strand breaks may play a role in mode of action of Cr VI. Double strand breaks in DNA were detected in HeLa cells incubated with hexavalent chromium using a comet assay and an anti-phospho-H2AX antibody (22). Activation of the protein kinase ataxia telangiectasia mutated (ATM), which occurs in response to DNA double strand breaks, was also observed in the HeLa cells treated with Cr VI. Exposure of *Drosophila melanogaster* larva to hexavalent chromium revealed a pattern of gene expression consistent with somatic recombination due to DNA double strand breaks (23).

The relative contribution of the formed reactive species to the DNA damage is unknown (9–11). Additionally, the newly formed trivalent chromium

may accumulate to high concentrations within the cell, and may be itself an important mediator of chromium carcinogenicity (24). Trivalent chromium has been shown to bind to isolated nuclei and DNA, and to cause DNA-protein crosslinks (10). These properties of rapid uptake into cells and intracellular generation of free radicals in the course of reduction to the directly genotoxic trivalent state, have led to the characterization of hexavalent chromium as a compound that "functions as a sort of Trojan horse" (6). It is widely believed that DNA damage from hexavalent chromium is a result of intracellular reduction, whereas extracellular reduction is considered a detoxification process (10–11). The contribution of reductive enzymes within the cell to the overall reduction of hexavalent chromium and DNA damage is not well understood (11).

The postulated mechanisms of hexavalent chromium-induced DNA damage include: 1) indirect free radical DNA damage, 2) direct metal-mediated oxidative DNA damage, and 3) direct metal-DNA binding. In support of the first mechanism is extensive evidence that suggests that reactive oxygen species, especially hydroxy radicals, and other free radical species are involved in the genotoxicity of hexavalent chromium (reviewed in 9–11). This evidence includes the measurement of reactive oxygen species in *in vitro* tests of hexavalent chromium genotoxicity, observations of lesions consistent with damage caused by reactive oxygen species and other free radicals (e.g., oxidized DNA bases, abasic sites, DNA strand breaks, and DNA-DNA and DNA-protein crosslinks) following hexavalent chromium treatment *in vitro* and *in vivo*, and observations that hexavalent chromium toxicity is reduced in the presence of free radical scavengers (reviewed in 5, 11).

The second mechanism, as proposed by Sugden and Stearns, involves DNA damage via direct metal-mediated oxidation (11). This mechanism stems from studies of DNA oxidation using model Cr (V) complexes. It is consistent with observations that treatment of human lung cells with hexavalent chromium results in expression of general oxidant-stress genes, but not in the expression of stress genes that respond to oxygen radicals. Also, the mutational spectra in cells treated with hexavalent chromium is different from the mutational spectra in cells treated with hydrogen peroxide, dioxygen or X-rays. This mechanism is also consistent with observations that hexavalent chromium reduction by ascorbate, glutathione or hydrogen peroxide was not accompanied by oxygen-radical formation.

In support of the third mechanism, researchers have observed direct binding of chromium with DNA and other cellular macromolecules (reviewed in [5]). Chromium can interact with DNA to form chromium-DNA adducts and DNA-protein crosslinks, and it can interact through other means that can also result in interference with DNA replication. Such interactions can give rise to effects such as mutation, aneuploidy, or alteration of gene transcription (reviewed in 5, 10).

METABOLISM AND PHARMACOKINETICS

Substantial information regarding the toxicokinetics of chromium began to be collected in the 1950s as the result of the use of radiolabeled chromium as a marker for measuring red blood cell turnover in humans. In addition, impetus to investigate the toxicokinetics of chromium in humans and animals resulted from the well-known carcinogenic effects of inhaled hexavalent chromium. Much of the toxicokinetic research that was conducted to address inhalation exposure to hexavalent chromium is relevant to the evaluation of exposure to hexavalent chromium via the oral route.

In most studies, it is unclear which form(s) of chromium occurred in the tissues because most investigators did not employ methods aimed at differentiating between hexavalent and trivalent or other valences of the metal (total chromium levels are reported). Because of its reactivity, it is difficult to resolve which form(s) of the metal are actually present in a tissue unless specific analytical methods are used. However, since hexavalent but not trivalent chromium readily crosses biological membranes, the two forms of the metal behave differently in biological systems. Observed differences in behavior act as "fingerprints" that can be employed to identify the presence of a particular form of chromium in a tissue in studies that did not attempt to differentiate which form(s) were present.

Reduction of Hexavalent Chromium to Trivalent Chromium

The ability of the stomach and other organs to reduce hexavalent chromium to trivalent chromium has previously been discussed in detail (7, 8, 25). Suffice it to say that *in vitro* and *in vivo* studies in animals and humans have demonstrated rapid reduction of hexavalent chromium to trivalent chromium. It should not be concluded from these studies, however, that all hexavalent chromium is reduced before it can be absorbed, as will be discussed in the following sections.

Absorption

Trivalent chromium is poorly absorbed from the gastrointestinal tract. Only a few percent (at most) of an orally administered dose of trivalent chromium appears to be absorbed in humans or experimental animals. Typically, one percent or less of an orally administered dose of trivalent chromium is recovered in the urine of experimental animals (26, 27) or humans (27–30).

Oral absorption of trivalent chromium does not appear to appreciably increase when the metal is complexed with an organic ligand (30–32). Infusion of trivalent chromium into the duodenum or jejunum resulted in at most one to two percent of the dose being absorbed in humans (27), or one percent (26) to

four percent (27) in the rat. Thus, bypassing the stomach and the reductive capacity of gastric components did not noticeably increase the amount of trivalent chromium absorbed from the gut.

Only a small portion of an orally administered dose of hexavalent chromium appears to be absorbed from the gut. The amount of chromium recovered in the urine was below ten percent of the administered dose of hexavalent chromium in humans, 6.9% (28), 3.4% (33), 1 to 4% (34), 2% (35); or in the rat, 2% (26). This is probably due to the substantial reduction of hexavalent chromium to trivalent chromium in the stomach. But studies in humans and the rat do indicate a greater absorption of hexavalent chromium than trivalent chromium (26–28, 33).

Infusion of hexavalent chromium into the duodenum or jejunum resulted in marked increases in absorption in humans (27) and experimental animals (26, 27). Donaldson and Barreras recovered 11 to 30% of the administered dose of hexavalent chromium in human urine when the metal was infused into the intestine (27). Only 57% of the dose of hexavalent chromium administered into the ligated jejunum of rats was recovered in the jejunum after sixty minutes (26). Approximately 98% of the dose of trivalent chromium was recovered in the jejunum under the same experimental conditions. Following the oral administration of hexavalent chromium to humans, increased recovery of chromium in the urine was observed under conditions of low stomach acidity (pernicious anemia) compared to control (eight percent vs. two percent) (27).

Kerger and associates administered hexavalent chromium admixed in an acidic orange juice vehicle to determine to what degree the acidic-organic environment (somewhat analogous to the stomach) reduces the oral absorption of the metal (28). The addition of hexavalent chromium to orange juice prior to its ingestion was a de facto pretreatment (reductive) of hexavalent chromium. Yet, in spite of pretreatment, the fraction of the administered dose of chromium recovered in the urine appeared to be greater (0.6%) than when trivalent chromium was administered (0.13%). However, the fraction was considerably less than when hexavalent chromium was administered in water (6.9%).

Distribution

When hexavalent chromium is incubated with washed isolated red blood cells (RBC), almost the entire dose is taken up by the cells. In contrast, little trivalent chromium appears to be taken up by RBC in *in vitro* incubations (27, 36–38). When hexavalent chromium is incubated with whole blood or RBC plus plasma, only a fraction (depending on conditions) of the hexavalent chromium is taken up by the RBC (39–42). This is probably due to the reduction of a portion of the administered hexavalent chromium to trivalent chromium outside of the RBC (43–45). The converted trivalent component of chromium is then largely excluded from the RBC. When hexavalent chromium was inhaled or

administered intratracheally, intraperitoneally, or intravenously, much of the chromium in the blood (25 to 70%) was taken up by RBC (46–51). At the same time, a sizable portion of chromium in blood remained in the plasma fraction (30 to 75%), probably because it had been reduced to trivalent chromium (39, 44, 45, 52, 53).

While negligible amounts of trivalent chromium were associated with RBC in many *in vivo* studies (40, 47, 48, 50, 54–56), in other studies there is some evidence that trivalent chromium is associated with the RBC, particularly at higher concentrations (39, 46, 55, 57–59). A large portion of the trivalent chromium associated with the RBC fraction typically can be washed free, implying it is loosely bound to sites on the outside of the RBC (39). Although the amount of trivalent chromium that associates with the RBC appears to be substantially less than hexavalent chromium, it could be noticeable when a large dose of trivalent chromium is administered or when hexavalent chromium is mostly absent.

While most of the radiolabeled hexavalent chromium that is taken up by the RBC remains there for the RBCs' lifetime, a portion of the radiolabel is eluted. When *in vitro* labeled RBC are reinjected into their donors, about two percent of the labeled chromium is lost from the RBC during the first 24 hours. This is followed by a steady rate of elution or "leakage" of chromium from cells at a rate of about one percent a day (60). This leakage must be accounted for when determining the survival rates of RBC clinically. The International Committee for Standardization in Haematology (ISCH) developed a correction table that accounts for the elution of chromium from the RBC, facilitating more accurate estimates of RBC survival rates (60).

The ability of hexavalent chromium to penetrate the cell membrane is believed to be due to its uptake through anion channels in the plasma membrane. It should be noted that the structures responsible for the uptake of hexavalent chromium into RBC are present in other cells. Therefore, other cells would be expected to take up hexavalent chromium readily, while little trivalent chromium would be expected to be taken up by most cells. Indeed, oral, intratracheal, intravenous, or intraperitoneal administration of hexavalent chromium results in increased chromium levels in a number of tissues, while little uptake occurs following the administration of trivalent chromium (48, 51, 61–63). The uptake of hexavalent chromium was very rapid in the isolated perfused rat liver (64). Relative to hexavalent chromium, little uptake of trivalent chromium occurred even when it was administered intravenously, which ensured that the metal was immediately available for tissue and cellular uptake (50, 61, 65).

The widespread distribution of chromium into tissues following hexavalent chromium administration indicates that although reduction is likely to be occurring in the blood, it does not necessarily occur at a fast enough rate to prevent hexavalent chromium from reaching and being taken up by tissues. While chromium was detected in the kidney, spleen, RBC, and liver when

hexavalent chromium was administered, little chromium was detected in tissues from these organs following the administration of trivalent chromium except at the site of its excretion, the kidney (and at much lower levels than when hexavalent chromium was administered) (24, 49, 55, 62, 66). Uptake of hexavalent chromium by the liver is also indicated by elevated levels of chromium in the bile following intravenous administration of hexavalent chromium, compared to when trivalent chromium was administered (53, 67, 68).

Oral administration of hexavalent chromium revealed a slightly different pattern of distribution compared to other exposure routes, with high levels of chromium in the liver, spleen, and kidney but much lower levels in the RBC (69–71). Higher levels of chromium in the liver are consistent with the passage of blood from the gut directly to the liver. Little chromium was detected in tissues from these organs following oral administration of trivalent chromium.

In humans, there have been no direct observations on the distribution of the absorbed chromium in the body. However, the findings of studies discussed below suggest that patterns observed in animals also occur in humans. The human urinary half-lives of chromium following the oral administration of hexavalent and trivalent chromium are quite different, with an average half-life of 10 hours following trivalent chromium administration versus an average half-life of 39 hours following the administration of hexavalent chromium (28). The prolonged urinary half-life following hexavalent chromium administration suggests that there is a pool(s) of chromium that is slowly being released. This release or elution is reminiscent of the slow release of chromium from RBC that is routinely observed when labeled RBC are introduced into humans in nuclear medicine (60). However, the half-life of chromium associated with RBCs was quite short after oral administration of hexavalent chromium (28), indicating that this was probably trivalent chromium externally bound to the RBCs.

The prolonged urinary half-life after oral administration of hexavalent chromium appears consistent with oral studies in animals which showed elevated chromium levels in the liver, kidney, and spleen, while RBC and plasma chromium levels were only modestly elevated (24, 70, 71). Accumulation of hexavalent chromium in the liver following intravenous administration (50) also suggests that liver is an important site of hexavalent chromium uptake. The half-life of chromium in various organs (other than plasma) of rats administered hexavalent chromium exceeded 20 days (49). The slow release (elution or "leakage") of chromium from the liver and other organs in humans would explain the prolonged urinary half-life observed by Kerger and associates (28). The uptake of chromium into these organs after administration of hexavalent chromium would be consistent with the behavior of hexavalent, but not trivalent chromium.

Kerger and associates also administered hexavalent chromium admixed in an acidic orange juice vehicle to human volunteers to determine to what degree the acidic-organic environment (somewhat analogous to the stomach) reduces

the metal (28). Under these conditions, the urinary half-life was still prolonged (fifteen hours versus ten hours for trivalent chromium controls). These findings provide additional evidence that chromate is not completely reduced to trivalent chromium in the acidic conditions in the stomach.

For some subjects in the human studies, changes in chromium RBC levels behaved as if trivalent chromium had been administered (28, 34, 35). RBC and plasma chromium levels remained essentially unchanged, which probably reflected conversion of most of the hexavalent chromium to trivalent chromium in the stomach. In other individuals, increases in plasma and RBC chromium levels were observed. This difference probably reflects high variability in the amount of reduction of hexavalent to trivalent chromium in the human stomach. In some of the subjects with elevated chromium blood levels, chromium levels in the RBC fraction rose and declined rapidly. Most of the absorbed chromium appeared to have been reduced to trivalent chromium prior to associating with the RBC. However, chromium RBC levels did remain elevated in other individuals, indicating hexavalent chromium had penetrated the RBC membrane.

Elimination

Administered trivalent chromium is rapidly cleared from the blood, RBC, and plasma (46, 50, 56). Rapid declines of urinary chromium levels have also been observed (32). By contrast, following administration of hexavalent chromium, RBC chromium levels or the ratio of RBC to plasma chromium either did not decline as rapidly or remained elevated for quite some time (40, 46, 49, 50, 55, 72) compared to trivalent chromium.

The decline in chromium RBC levels following hexavalent chromium administration appears to be more rapid than what would be accounted for by the lifespan of the RBC. However, chromium-labeled RBCs prepared *in vitro* that are used in nuclear medicine are carefully washed so that hexavalent chromium is rapidly taken up by the cells and little is converted to trivalent chromium outside the cell. In contrast, the administration of hexavalent chromium by the oral route in the rat (40) and humans (28, 34, 35) most assuredly results in its rapid conversion to trivalent chromium outside the cell, which will associate with the outside of the RBC. Thus, following the oral administration of hexavalent chromium, the rapid decline in RBC chromium levels appears to reflect the predominance of trivalent chromium, as expected from the reduction of most of the dose in the stomach and plasma.

Two models are being proposed that describe the toxicokinetics of hexavalent and trivalent chromium. The models account for the differences in behavior of hexavalent and trivalent chromium observed in animals and humans (Figure 1). The increase in absorption, as reflected by increased plasma and erythrocyte levels, increased amount excreted in the urine, and prolonged plasma

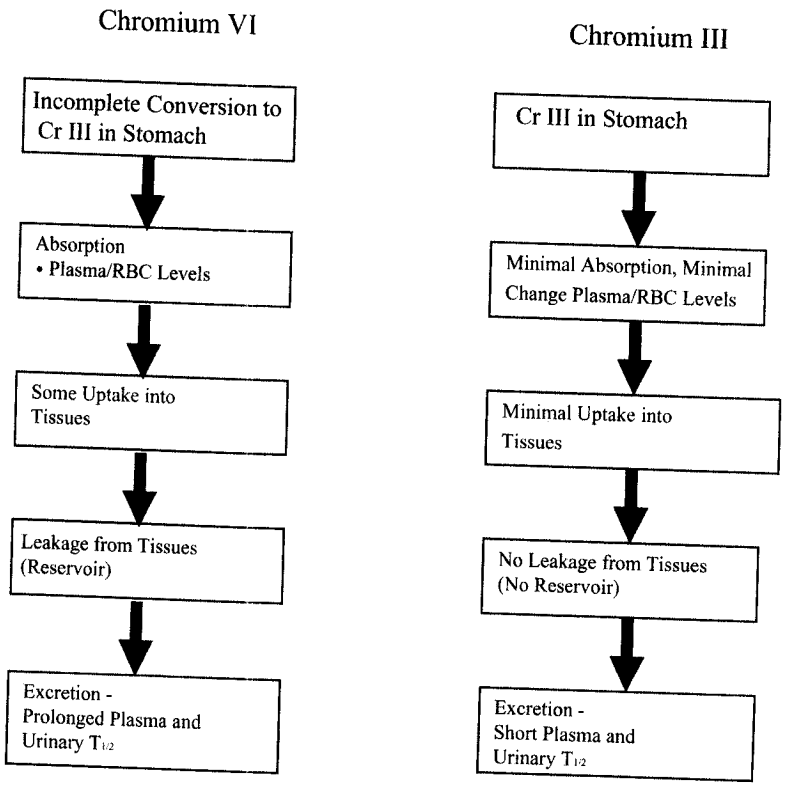

Figure 1: Toxicokinetic model—hexavalent chromium verses trivalent chromium.

and urinary half-lives, appears to indicate that the hexavalent form of the metal is orally absorbed, distributed to various organs and then taken up by cells. Based on the findings in animals, the liver is likely to be an important site of cellular uptake of hexavalent chromium (24, 69–71). The prolonged plasma and urinary half-life appear to result from chromium being taken up and then eluted from cells. The behavior of administered trivalent chromium—low plasma, erythrocyte and urinary levels, rapid decreases in plasma and erythrocyte levels, and short urinary half-life—indicate that this form of the metal is not appreciably absorbed from the gut and the small fraction that is absorbed is largely excluded from cells.

Toxicity

Hexavalent chromium appears to be more toxic than trivalent chromium, which appears to be non-toxic when administered by the oral route. No toxicity in the liver or other organs has been detected following administration of trivalent chromium to animals (73–75). However, as mentioned earlier, trivalent chromium may not be completely innocuous as many believe, as it has been

shown to bind to isolated nuclei and DNA and to cause DNA-protein crosslinks (10).

Studies conducted by NTP in which hexavalent chromium was administered in the feed resulted in effects in the liver (cytoplasmic vacuole formation) and blood forming organs (decreased mean corpuscular volume and mean corpuscular hemoglobin levels) (76). Two 22-week studies in male and female rats revealed vacuolation, degeneration and necrosis in the liver (77, 78). These differences in toxicity of hexavalent and trivalent chromium are consistent with toxicokinetic findings that hexavalent chromium is orally absorbed and is entering cells. These results provide important evidence that a significant fraction of hexavalent chromium is not being converted to trivalent chromium in the acidic environment of the stomach.

Genetic Toxicity

The genotoxic potential of hexavalent chromium compounds has been evaluated in short-term test systems, in animals *in vivo*, and in occupationally exposed workers (1). Hexavalent chromium is genotoxic without exogenous activation in bacteria, and in human and other mammalian cells in culture (reviewed in IARC (1), ATSDR (5), De Flora (79)). Hexavalent chromium compounds induced gene mutations in multiple species and strains of bacteria, and gene mutations, DNA-protein crosslinks, DNA strand breaks, chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis, and other forms of DNA damage in mammalian cells *in vitro*.

While the genotoxicity of hexavalent chromium compounds associated with *in vivo* exposures of humans and animals has been reviewed elsewhere (1, 5, 79), several new studies have been published in the scientific literature. The following summarizes the evidence of genotoxicity of hexavalent chromium, with an emphasis on those genotoxicity studies that employed an oral route of exposure, since the goal of this assessment is to determine the potential cancer health risk posed by hexavalent chromium in drinking water supplies.

Inhalation, Intratracheal, Intraperitoneal and Intravenous Exposures

IARC reviewed the studies of DNA damage in peripheral blood lymphocytes of workers exposed to hexavalent chromium, primarily by inhalation (1). IARC noted that "elevated levels of sister chromatid exchange were observed in workers exposed to hexavalent chromium compounds in electroplating factories in four out of six studies. Chromosomal aberrations were found in all three studies of exposed workers." Relatively few *in vivo* genotoxicity studies of hexavalent chromium following exposures to the respiratory system were located (80–82).

Cheng and coworkers administered to C57Bl/6 Big Blue mice (a strain containing the *lacI* reporter transgene) a single dose (6.75 mg/kg) of an aqueous solution of potassium chromate in the trachea (82). Mutation frequency in the *lacI* gene relative to background rates was significantly elevated in the lung and kidney ($p < 0.001$) and marginally significant in the liver ($p = 0.085$). The mutation frequencies in the lung, kidney and liver correlated closely with the concentration of chromium deposited in these organs (82). Izzotti and associates dosed Sprague-Dawley rats with intratracheal instillations of sodium dichromate (0.25 mg/kg) for three consecutive days, and observed increases in DNA fragmentation, DNA-protein crosslinks and oxidized DNA bases in the lung, but not the liver (81).

Data from these inhalation and intratracheal studies suggest that the greatest degree of DNA damage occurs in the respiratory tract (i.e., the portal of entry), and some smaller amount of DNA damage occurs at distant tissues following absorption of chromium by the lungs and distribution to those tissues. Some have argued that DNA damage in the lung and distant tissues will only occur above some threshold dose (6, 81).

Over 15 genotoxicity studies in which rodents were administered soluble hexavalent chromium compounds (e.g., sodium dichromate, potassium dichromate, potassium chromate) either intraperitoneally (i.p.) or intravenously (i.v.) were reviewed by De Flora (79), IARC (1), and ATSDR (5). The majority of the studies reported positive genotoxicity in tissues distant to the site of administration. No genotoxicity studies employing subcutaneous or intramuscular injection were described in the published reviews. In rodents administered hexavalent chromium via i.p. injection, significant increases were observed in mutations of the bone marrow and liver; chromosomal aberrations, micronuclei and sister chromatid exchanges of the bone marrow, polychromatic erythrocytes or lymphocytes; DNA single strand breaks of the liver; and DNA-protein crosslinks of the liver, lung and kidney (as reviewed by IARC (1), ATSDR (5), De Flora (79). In rodents administered hexavalent chromium compounds via i.v. injection, significant increases in chromosomal aberrations in bone marrow and lymphocytes were reported (as reviewed by De Flora (79)).

Oral Exposures

Thirteen primary studies of potential genotoxic effects following ingestion of hexavalent chromium by humans or other mammalian species were located (80, 83–94). A summary of these studies is provided in Table 1. Ten of the 13 studies reported positive genotoxicity findings in various tissues. Chromosomal aberrations of the bone marrow, DNA single strand breaks or DNA fragmentation of the liver and brain, or DNA-protein crosslinks of the liver were observed following exposure of rodents via drinking water or via chronic dosing by gavage. Surprisingly, no study to date has looked for DNA damage in the oral cavity

Table 1: Summary of *in vivo* genotoxicity studies of hexavalent chromium by the oral route.

Study	Species	Vehicle	Dose and dose regimen	Response	Genotoxic endpoint and site[1]
Bigaliev et al. (77)	White rats	Gavage[2]	1 mg/kg-d, potassium dichromate, one year	+	Chromosomal aberrations in bone marrow
	White rats	Gavage	15 mg/kg, potassium dichromate, single dose, measured 2, 4, 6, 8 or 12 hr after dosing	+	Chromosomal aberrations in bone marrow
Shindo et al. (80)	MS/Ae mice	Gavage	20 to 320 mg/kg, potassium chromate, single dose, measured 24 hr after dosing	–	Micronuclei in polychromatic erythrocytes
	CD-1 mice	Gavage	20 to 320 mg/kg, potassium chromate, single dose, measured 24 hr after dosing	–	Micronuclei in polychromatic erythrocytes
Coogan et al. (81)	F344 rats	Drinking water	100 or 200 ppm (6.1 or 8.7 mg/kg-d) potassium chromate, three weeks	+	DNA-protein crosslinks in liver
				–	DNA-protein crosslinks in lymphocytes
Sarkar et al. (82)	Swiss mice	Gavage	20 mg/kg, chromium(VI) oxide, single dose, measured 24 hr after dosing	+	Chromosomal aberrations in bone marrow
Bagchi et al. (83)	Sprague-Dawley rats	Gavage	10 mg/kg-d, sodium dichromate, 15, 30, 45, 60, 75 or 90 days	+	DNA single strand breaks in liver
Bagchi et al. (84)	Sprague-Dawley rats	Gavage	25 mg/kg, sodium dichromate, single dose, measured 48 hr after dosing	+	DNA single strand breaks in liver
Bagchi et al. (88); Bagchi et al. (89); Bagchi et al. (90)	C57BL/6Ntac and p53 deficient-C57BL/6TSG p53 mice	Gavage	1.9, 19 or 95 mg/kg, sodium dichromate, single dose, measured 24 hr after dosing	+	DNA fragmentation in liver and brain
Bagchi et al. (85)	Sprague-Dawley rats	Gavage	2.5 mg/kg-d, sodium dichromate, 120 days	+	DNA single strand breaks in liver and brain
Dana Devi et al. (91)	Swiss Albino mice	Gavage	0.59, 1.19, 2.38, 4.75, 9.5, 19, 38 or 76 mg/kg, single dose, measured 1, 2, 3, 4, 7, and 14 day after dosing.	+	Single- and double-stranded DNA breaks in leukocytes.
Kuykendall et al. (86)	Humans	Drinking water	5 mg (~0.007 mg/kg), potassium dichromate, in 0.5 L water	–	DNA-protein crosslinks in leukocytes
Mirsalis et al. (87)	Swiss-Webster mice	Drinking water	1 to 20 ppm (~0.2 to 3.5 mg/kg-d) potassium dichromate, two days, measured 24 hr after dosing	–	Micronuclei in polychromatic erythrocytes
	Swiss-Webster mice	Gavage	0.02 to 0.4 mg/kg, potassium dichromate, two days, measured 24 hr after dosing	–	Micronuclei in polychromatic erythrocytes
	F344 rats	Drinking water	1 to 20 ppm (~0.05 to 1.0 mg/kg-d) potassium dichromate, two days, measured 24 hr after dosing	–	Micronuclei in polychromatic erythrocytes

[1]It is important to note that no group has looked for genotoxicity of the oral cavity or gastrointestinal tract following oral administration of hexavalent chromium.

[2]In the Bigaliev et al. (87) study, for this dose group only, the methods translated from Russian state that the rats were chronically administered with a "...dosage 1 mg per 1 kg of live weight orally or inside trachea with 0.2 mL of 5% solution of $K_2Cr_2O_7$." It is difficult to interpret this statement, but it appears that the authors were not sure to what extent the dosing tube was passed into the stomach or the trachea over the year-long dosing period.

or gastrointestinal tract following oral administration of hexavalent chromium. The data are consistent with the idea that, following low to moderate bolus doses (gavage) or higher concentrations in drinking water, hexavalent chromium is absorbed by the intestines and is transported to distant tissues where it damages DNA. Studies of genotoxicity of the oral cavity and gastrointestinal tract following oral ingestion of hexavalent chromium are needed.

The three oral genotoxicity studies that reported negative findings each employed short-term exposures: Shindo and coworkers administered a single dose by gavage (83); Kuykendall and associates administered a single dose in 0.5 L of drinking water (89); and Mirsalis et al. administered either two doses by gavage or dosed the animals via drinking water over a two-day period (90). One study assayed for DNA-protein crosslinks in leukocytes (89). The usefulness of this endpoint as a sensitive biomarker of hexavalent chromium exposure has been called into question (84, 95). However, four studies that employed a single oral dose of hexavalent chromium in mice yielded increases in DNA fragmentation in the liver and brain (91–93), or DNA strand breaks in leukocytes (94). These genotoxic effects peaked at 48 hours after dosing. Both the timing of the exposure and the endpoint assessed appeared to influence the occurrence of genotoxicity.

There is some concern that high doses of hexavalent chromium, such as those received by oral gavage or by rapidly drinking a large glass of contaminated water, may overwhelm the reducing capacity of the stomach. Indeed, the reductive capacity of the oral cavity and stomach and the dose rate at which hexavalent chromium is ingested are important factors to consider in determining risk. Based on these data, De Flora suggests that the saliva and stomach have the capacity to completely reduce the dose that a human would receive from rapid ingestion of hexavalent chromium-containing drinking water at concentrations typically found in California water supplies (6). However, genotoxic effects in distant tissues (i.e., bone marrow, liver and brain) have been observed in rodents chronically administered hexavalent chromium by gavage at doses that are not likely to overwhelm the reductive capacities of the stomach, intestines and blood: 0.59 mg/kg (94); 1.0 mg/kg-day (80); 1.9 mg/kg (93); 2.5 mg/kg-day (88).

CANCER STUDIES

Human exposure to hexavalent chromium by the inhalation route has been linked to increased rates of cancer in several occupational studies. A number of retrospective studies have associated significant increases in respiratory cancer to hexavalent chromium exposure in workers engaged in chromate production and chromate pigment production (1). Increased incidence of lung cancer has also been observed in workers employed in the chromium plating industries.

Because the evidence on carcinogenic effects of hexavalent chromium has been summarized by others, principally for the inhalation route (1), we focus on the evidence of systemic availability and the resulting risk of carcinogenic effects after oral exposure.

Animal Studies

Only one animal carcinogenicity study, Borneff et al. (96), was identified in which hexavalent chromium was administered by the oral route for the lifetime of the animal. An additional short-term study of skin tumor formation following exposure of mice to hexavalent chromium in drinking water and/or ultraviolet light was reported by Davidson and coworkers (97). Using a three-generation study design, Borneff et al. treated 120 female and 10 male NMRI mice with 1 mg K_2CrO_4 per day (500 ppm) in drinking water (containing 3% household detergent). An equal number of animals received drinking water (3% detergent) only. In addition, two groups of 120 females and 10 males which received either benzo[*a*]pyrene alone or benzo[a]pyrene + 500 ppm K_2CrO_4 in drinking water were included in the study. Animals were mated six weeks after the start of treatment. Two mice from each litter were selected as the first generation (F_1) mice. Three weeks after birth these mice were separated by sex and received the same food and concentration of test substance [0 or 500 ppm K_2CrO_4, or benzo[*a*]pyrene or benzo[*a*]pyrene + 500 ppm K_2CrO_4] in their drinking water as did the parent (F_0) generation. An outbreak of mousepox (ectromelia) virus occurred during the eighth month of the experiment and a majority (512) of the animals died. All surviving animals received a mousepox vaccination two months after the outbreak, which effectively ended the epidemic. In the F_0 generation, 41 of 130 mice (male and female) receiving K_2CrO_4 and 56 of 130 of the control mice survived the epidemic and are considered to be at risk.

First generation (F_1) mice were mated after the mousepox epidemic had ended. There were fewer offspring from the mating of F_1 mice than after the breeding of the F_0 animals. The F_2 generation mice received the same food and concentration of test substance [0 or 500 ppm K_2CrO_4, or benzo[*a*]pyrene or benzo[*a*]pyrene + 500 ppm K_2CrO_4] in their drinking water as did the F_0 and F_1 generations. The F_2 mice received the pox vaccine at two months of age, and all animals received a second dose of the vaccine three months later. These studies were terminated after 880 days. At the time of termination, F_2 mice had been exposed for approximately 17 months (510 days).

Combined forestomach tumors in the three generations of mice underlie the statistical analysis of this study, although most of the tumors occurred in the first generation. Two carcinomas of the stomach were observed in female mice exposed to K_2CrO_4. No malignant stomach tumors were found in control mice. Nine benign stomach tumors were observed in female mice exposed to K_2CrO_4. The increase in malignant and benign tumors in animals administered

hexavalent chromium (11/66) was statistically significant in comparison to the control group (2/79).

The study had both strengths and weaknesses. The study contained both vehicle and positive control groups and a large number of female mice per treatment group. The animals were exposed to hexavalent chromium in drinking water for their lifetime, and the drinking water solution containing K_2CrO_4 was analyzed at regular intervals to confirm its stability.

The high early mortality in the F_0 generation that occurred as the result of the mousepox epidemic is a concern because it could have compromised the ability of the study to detect a carcinogenic response or have been responsible for the tumors. Fortunately, because the study began with rather large numbers of animals, an adequate number of animals survived to allow sufficient sensitivity to detect a carcinogenic response. Also, there is no evidence that the increase in tumors observed in female mice was due to the virus. There is no evidence that the forestomach of the mouse is a site where mousepox lesions occur (98). If these lesions resulted from the mousepox infection, then an equal increase in papillomas should have been observed in "surfactant only" vehicle control animals, which did not occur.

Only one dose level of hexavalent chromium was employed in the study, which could have been excessive (above the maximum tolerated dose). Borneff and associates stated that the dose chosen was "close to the maximum concentration that is tolerated by mice without developing any damage" (96). The paper did not report any toxicity, excess mortality, or weight loss associated with K_2CrO_4 treatment. Given the lack of toxicity, the dose administered did not appear to be excessive or to exceed the maximum tolerated dose (MTD) (99–103).

There were also no reported preneoplastic lesions in the forestomach of mice administered hexavalent chromium in this study. However, no preneoplastic lesions were observed in mice administered benzo[*a*]pyrene, whose administration resulted in significant increases in tumors of the forestomach in this study and in previous studies by Borneff and coworkers (104) and other investigators (105). Thus, the investigators either did not observe or may not have reported preneoplastic lesions in the forestomachs of mice treated with either hexavalent chromium or benzo[*a*]pyrene.

In the short-term cancer study by Davidson and associates, groups of 6-week old hairless SK1-hrBR mice (20 animals per group) were exposed to potassium chromate and/or UV light and observed for skin tumor formation (97). The exposure groups were as follows: controls (Group 1), UV radiation only (Group 2), 2.5 ppm K_2CrO_4 (Group 3), 5.0 ppm K_2CrO_4 (Group 4), UV + 0.5 ppm K_2CrO_4 (Group 5), UV + 2.5 ppm K_2CrO_4 (Group 6), and UV + 5.0 ppm K_2CrO_4 (Group 7). Cr^{+6} was administered in the drinking water for 182 days. UV light exposures (1.18 kJ/m^2) were begun after the first month of Cr^{+6} treatment at a frequency of 3 days per week and continued for three months. After a

1-week break, UV treatments resumed for 3 additional months at a frequency of 2 days per week. Animals were sacrificed at approximately 224 days of age. No skin tumors were observed among controls or mice treated only with Cr^{+6} (Groups 1, 3 and 4). However, co-exposure of UV and Cr^{+6} resulted in skin tumor formation that demonstrated a clear dose-response increase with increasing concentration of Cr^{+6} (Groups 2, 5, 6, and 7). Since many humans are exposed to both UV radiation from sunlight and hexavalent chromium in drinking water, the authors concluded that the findings support concern over the potential carcinogenic hazards posed by hexavalent chromium in drinking water.

Human Studies

Drinking water exposure

Only one study was identified in which cancer risk was investigated in a population demonstrably exposed to hexavalent chromium in drinking water. Zhang and Li studied lung cancer and stomach cancer in rural villages near JinZhou, China, and reported increased stomach cancer rates associated with hexavalent chromium exposure (106). Another report suggested that the increase in cancer was unrelated to the exposure to chromium in drinking water (107). We re-evaluated the findings in the original reports as well in four additional reports (108–111) that were prepared by the investigators (Beaumont et al., in preparation).

The source of the contamination was a chromium ore smelting facility located in a rural area near the city of JinZhou in Liaoning Province in northeastern China. Hexavalent chromium contamination was detected in area wells in 1965. The date when substantial releases of hexavalent chromium commenced is unclear. Full-scale production which began in 1965 was associated with dramatic increased releases of production wastes (110). The releases were reportedly not fully controlled until 1980–1982, when a concrete anti-migration wall was installed around the site.

Groundwater from wells in two villages near the plant began to appear yellow (contaminated) in 1965. The movement of groundwater contamination appeared to be rapid and by the end of 1965, groundwater contamination had expanded to approximately half (41%) of the wells in the nearest village and 96% of the wells in the second nearest village. Hexavalent chromium also began to be detected in wells in the other villages in 1965.

Health morbidity surveys found evidence of exposure in the villages. A survey of residents in Nuer River Village in 1965 revealed mouth ulcer, diarrhea, stomach pains, indigestion and vomiting, symptoms associated with oral exposure to hexavalent chromium (5). In 1971, a survey of subjects in the second farthest village from the plant revealed 92% developed oral ulcers, 48% had

diarrhea, and 36% had abdominal pains. These symptoms were observed in 1974 in the most remote of the five villages near the alloy factory.

The investigators reported that after various mitigation measures were undertaken, hexavalent chromium concentration in the underground water at the plant started to decrease quickly after 1967 and appeared to become stable for many years (110). They also said that: "In 1974, the contaminated area had expanded into a region of about 45 square kilometers." A report from the Chinese government in 1994 stated: "... a chromium residue disposal site in JinZhou caused ground water pollution in a 12.5 km^2 area; as a result, water from 1,800 wells in nine villages is no longer potable" (112).

The detection of high levels of hexavalent chromium in groundwater samples does not necessarily indicate that all of the population in the area was exposed to high chromium levels. There was indication that some of the residents obtained alternative sources of drinking water. The investigators stated that: "In early 1970s, a system to provide tap water to all the residents in this region was gradually installed" (110). It is unclear which residents were provided drinking water (i.e., what "in the region" precisely means) and when this occurred. It is likely that residents with the most unpalatable well water were provided an alternative source.

A paper published in 1997 noted that the villages closest to the plant with higher levels of hexavalent chromium in drinking water in 1965 had lower cancer rates than villages with lower levels of hexavalent chromium, and concluded that the risk of cancer was probably unrelated to exposure to hexavalent chromium (107). However, based on the recently available reports from China, this conclusion does not appear to be credible. First, it did not address the actual pattern of exposures to hexavalent chromium during the entire period. In villages nearest the contamination, the water from some of the wells became essentially unpalatable in 1985 and was not necessarily consumed, while populations down gradient may have continued to drink the well water. Second, the proportion of wells contaminated in each village (and the proportion of people exposed) is likely to have increased as the plume spread out down gradient. Third, the reduction of contamination at the source may have resulted in a peak of the contaminant moving down gradient over the study period. This pattern would be consistent with elevated levels of cancer in the more distant villages.

Given the uncertainties regarding the levels of hexavalent chromium in groundwater after 1965, no conclusions are warranted concerning whether certain villages were exposed to more hexavalent chromium than other villages. Therefore, proximity to the alloy plant should not be considered as a surrogate for exposure level.

Because it is unclear if any of the villages were exposed to higher levels of hexavalent chromium in their drinking water, OEHHA combined the population and cancer data for the five villages with documented hexavalent chromium drinking water contamination to form a single exposed population (Beaumont

Table 2: Rate ratios of cancers in villages with hexavalent chromium in their wells

Site	Rate ratios[a]
All sites	1.23, 0.97–1.53
Stomach	1.69, 1.12–2.44[b]
Lung	1.78, 1.03–2.87[b]

[a]Rate ratio, 95% confidence interval.
[b]Statistically significant ($p < 0.05$).

et al., in preparation). Rates for mortality from all cancer, stomach cancer, and lung cancer in the combined exposed villages were compared to the rates in Liaoning Province (in which the villages were located) by calculating rate ratios (rate in exposed villages combined/rate in province). Rates for the province adjusted to the 1964 age distribution of China were obtained from the *Atlas of Cancer Mortality in the People's Republic of China, rates for 1973–75* (113). Exact mid-P 95% confidence intervals and 2-sided hypothesis test probabilities were calculated for 70 or fewer deaths, and approximate Fisher's confidence intervals and probabilities for more than 70 deaths, using the PEPI Describe computer program for the Poisson distribution (114, 115).

The rate ratio (RR) for all cancers combined (Table 2) was elevated but not quite statistically significant ($p = 0.078$). The rate ratio for stomach cancer, however, was higher and statistically significant ($p = 0.013$). The rate ratio for lung cancer was similarly elevated, but of less statistical significance ($p = 0.039$) due to smaller numbers of deaths.

The Zhang and Li findings had several important limitations. The study employed an ecological epidemiological design, in which cancer rates in geographic areas were compared without data on exposure to individual residents. It is likely that not all persons in the villages classified as exposed were actually exposed to contaminated drinking water (not all wells were contaminated, and alternative sources of water were provided to certain residents), and, conversely, it is possible that villages and regions classified as unexposed had undocumented contamination of the drinking water.

Another limitation was lack of data on levels of airborne hexavalent chromium. The reports from the JinZhou Health and Anti-Epidemic Station (108–111) contained no data on air contamination, which may have occurred from dust from outdoor ore residue stacks and emissions from the production process. While the prevailing wind direction was to the north-northeast and the villages with contaminated water were to the east, it is possible that the villages with contaminated water experienced some exposure to airborne hexavalent chromium. A third limitation was sparse information regarding the magnitude and length of exposure to contaminated water. Another limitation was the study's relatively short observation time (13 years), which would limit the study's ability to detect increases in cancer. However, increases in stomach and lung cancers were detected in spite of this limitation. The study also did

not indicate if any of the villagers worked in the alloy plant and therefore could have received exposure to high levels of chromium presumably due to inhalation. However, the investigators did not detect a significant increase in lung tumors in areas near the plant.

While the study had substantial limitations, it is clear that hexavalent chromium was released from the alloy plant, that underground water became contaminated, and that the contaminated water was used as a source of drinking water in villages adjacent to the plant. Additional information resulting from a thorough groundwater hydrological investigation, information on which villages were provided alternative sources of drinking water, and information on the effectiveness of remedial measures could be employed to yield a more complete exposure analysis.

Occupational exposures

While inhalation is the primary route of exposure in occupational populations exposed to hexavalent chromium, particulates that deposit in the upper respiratory region are cleared by mucociliary action and then swallowed. Thus, a portion of inhaled hexavalent chromium could yield tumors in the oral cavity or gastrointestinal tract (non-respiratory cancers).

A preliminary analysis by OEHHA of occupational studies where exposure to hexavalent chromium occurred by the inhalation route suggests an increase of the risk of stomach cancer (Beaumont et al., in preparation). Meta-analysis of the results of the 16 studies of populations occupationally exposed to hexavalent chromium yielded an overall rate ratio of 1.30 (95% CI 1.11–1.53, $p = 0.002$) for stomach cancer. When the analysis was restricted to 10 studies that found a statistically significant excess risk of lung cancer (an indicator of substantial hexavalent chromium exposure), the overall rate ratio for stomach cancer was 1.35 (95% CI 1.13–1.62, $p = 0.001$).

DISCUSSION

Studies that have investigated the mechanism of action of hexavalent chromium suggest that once hexavalent chromium enters a cell, regardless of the route of exposure, there is a potential for genotoxicity and therefore for a carcinogenic response. While numerous studies have linked inhalation exposure to hexavalent chromium with an increased risk of lung cancer, its rapid reduction to trivalent chromium in the stomach has led some to conclude that hexavalent chromium in drinking water does not pose a carcinogenic risk.

Genotoxicity studies in animals administered hexavalent chromium by the oral route revealed DNA damage in tissues distal to the site of administration (the liver and blood forming cells). These findings suggest that a portion of orally administered hexavalent chromium enters cells. Toxicokinetic studies also indicate that a small fraction of an orally administered dose of hexavalent

chromium is not converted to trivalent chromium in the stomach. Orally administered hexavalent chromium in humans and animals behaves quite differently than orally administered trivalent chromium. A greater fraction of an oral dose of hexavalent chromium was recovered in the urine and higher blood and plasma chromium levels are observed following hexavalent chromium administration. In addition, administration of hexavalent chromium resulted in a prolonged plasma and urinary half-life in humans, compared to trivalent chromium.

Differences in the oral toxicity of hexavalent and trivalent chromium in animal studies buttress the findings that hexavalent chromium is not completely converted to trivalent chromium in the stomach. Only one epidemiological study was identified that specifically addressed human exposure to hexavalent chromium in drinking water (106). Our analysis found a statistically significant increase in stomach cancer in a population exposed to hexavalent chromium in drinking water. The amount of exposure of this population to hexavalent chromium is uncertain because the hexavalent chromium levels in the wells were poorly characterized. What is quite evident is that a portion of this population was exposed to high levels of hexavalent chromium.

Only one carcinogen bioassay was identified in which animals were exposed to hexavalent chromium in drinking water (96). A statistically significant increase in tumors of the forestomach was observed in the female mouse. There is uncertainty associated with this finding because of the occurrence of viral infection that caused substantial intercurrent mortality, the lack of reported preneoplastic lesions and by the inclusion of only one dose level. However, there is no evidence that the increase in tumors was due to the viral infection, or that other limitations of this study would invalidate the observed increase in stomach tumors in treated animals.

The findings of toxicokinetic, genotoxicity, and general toxicity studies as well as the available epidemiological and animal cancer bioassay results are not consistent with the assertion that hexavalent chromium is completely converted to trivalent chromium in the animal or human stomach. Taken together, the lines of evidence lead us to the conclusion that exposure to hexavalent chromium in drinking water should be considered to pose an increased risk of cancer to humans. How this conclusion should be incorporated into a risk assessment for oral exposure to hexavalent chromium remains an important issue.

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