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Mechanisms of Chromium Carcinogenicity and Toxicity

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ABSTRACT: Chromium, like many transition metal elements, is essential to life at low concentrations yet toxic to many systems at higher concentrations. In addition to the overt symptoms of acute chromium toxicity, delayed manifestations of chromium exposure become apparent by subsequent increases in the incidence of various human cancers. Chromium is widely used in numerous industrial processes, and as a result is a contaminant of many environmental systems. Chromium, in its myriad chemical forms and oxidation states, has been well studied in terms of its general chemistry and its interactions with biological molecules. However, the precise mechanisms by which chromium is both an essential metal and a carcinogen are not yet fully clear. The following review does not seek to embellish upon the proposed mechanisms of the toxic and carcinogenic actions of chromium, but rather provides a comprehensive review of these theories. The chemical nature of chromium compounds and how these properties impact upon the interactions of chromium with cellular and genetic targets, including animal and human hosts, are discussed.

KEY WORDS: DNA adducts, DNA protein cross-links, reductive intermediates.

I. INTRODUCTION

Chromium is believed to be an essential metal in small quantities, but also is recognized by IARC as a potent human carcinogen. Chromium carcinogenesis has been known since the late 19th century, when the first nasal tumors were described among Scottish chrome pigment workers. Epidemiological studies of carcinogenesis among industrially exposed chromium workers have since identified other chromium industries, such as chrome plating, leather tanning, and stainless steel production, as potential sources of human exposure to this metal. As a result of chromium use in industry, environmental contamination serves as an additional source of human exposure to chromium. This review briefly summarizes the current literature regarding chromium chemistry and biochemistry, and outlines the mechanisms by which chromium may be essential to life, but also is toxic, genotoxic, and carcinogenic in animal models and in humans. Chromium has been the subject of many written reviews in the recent past, and references to this extensive literature have been included in the appropriate sections of this article.

II. CHEMISTRY OF CHROMIUM AND ITS COMPOUNDS

Chromium ($[Kr]4s^13d^5$) is a first-series transition element from group VIB. In its elemental form, it is a white, hard, lustrous, and brittle metal with a fairly high melting point (approximating 2000°C). It can exist in oxidation states ranging from -2 to $+6$, but only the 0, $+2$, $+3$, and $+6$ forms are commonly encountered.

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Recent studies have identified complexes containing Cr(V) or Cr(IV), but these usually are not very stable or isolatable in large quantities.[1-9] The majority of chromium compounds is found as either halides, sulfides, or oxides (Table 1).[10] Conversely, hexavalent chromium is a strong oxidant ($E^\circ = +1.41$ V)[11-13] and exists primarily in the form of oxides.

Chromium(II) compounds exist primarily as high-spin distorted octahedral or tetrahedral structures; this malformation is the result of Jahn-Teller distortions. Divalent chromium is a strong reductant ($E^\circ = -0.41$ V) and rapidly decomposes in air or water to yield the inert trivalent species. Nearly all the thousands of Cr(III) complexes known are hexacoordinate. Trivalent chromium readily forms low-spin octahedral coordination compounds, complexes, and chelates. Its relative inertness is due to the stability of its half-filled outer electronic shell (t_{2g}); compared with many other first-row transition metals, its ligand binding and exchange rates are among the slowest (i.e., $t_{1/2}$ = hours at room temperature).[10,14] Trivalent chromium is considered a borderline hard acid metal and so forms bonds primarily with ionic ligands: the Cr(III) complex stability series follows the order $F^- > Cl^- > O^- > S^-$ ligands.[15] In nature, the majority of Cr(III) complexes exists as cationic hexaaqua salts, ammoniates, or as amines; direct complexation with halides, isothiocyanate, or cyanide gives rise to a few anionic compounds. Hydration of Cr(III) compounds results in olation by way of hydroxo ligands, and the formation of polynucleated bridged complexes; similarly, bridging can occur through amino- or oxo-links.[16]

Chromium(IV) and Cr(V) compounds are the least frequently occurring of all the chromium

TABLE 1
The Varied Oxidation States of Representative Chromium Compounds

Oxidation state	Outer orbital configuration	Examples
+0	$[Kr]4s^13d^5$	$Cr(CO)_6$, $Cr(bipy)_3$ Metallic chromium Several chromium alloys
+1	$[Kr]3d^5$	$[Cr(CNR)_6]^+$, $[Cr(bipy)_3]^+$
+2	$[Kr]3d^4$	$CrCl_2(MeCN)_2$, $CrCl_2$, CrS CrF_2, CrI_2, $CrBr_2$, $Cr_2[(CH_2)_2P(CH_3)_2]_4$
+3	$[Kr]3d^3$	Cr_2O_3, $CrCl_3$, $Cr(acac)_3$ CrF_3, $CrBr_3$, CrI_3, $CrPO_4$, $Cr(NO_3)_3$, $FeCr_2O_4$, $Cr_2(SO_4)_3$, $K_3[Cr(CN)_6]$, $[Cr(NH_3)_6]^{3+}$
+4	$[Kr]3d^2$	K_2CrF_6, $Cr(CH_2SiMe_3)_4$, Sr_2CrO_4, Ba_2CrO_4, CrO_2, Na_4CrO_4, $Cr(OC_4H_9)_4$, $CrCl_4$, CrF_4, $CrBr_4$
+5	$[Kr]3d^1$	CrO_4^{3-}, CrF_5, $CrOCl_4^-$, Li_3CrO_4, $Ca_3(CrO_4)_2$, K_3CrO_4
+6	$[Kr]3d^0$	Na_2CrO_4, K_2CrO_4, $SrCrO_4$, $ZnCrO_4$, $PbCrO_4$, $CaCrO_4$, CrO_2Cl_2, CrO_3, $Na_2Cr_2O_7$, $FeCr_2O_4$, CrF_6

species. They are relatively unstable and usually require special handling procedures.[10] Although the most common and most stable Cr(IV) agent known is the CrO_2 oxide, other stable tetravalent chromium compounds can be found primarily as mixed oxides with other metals (i.e., Ba, Sr, Si). Chromium(V) compounds are only slightly more stable. In general, the alkali and alkaline earth chromates(V) that are formed readily decompose to Cr(III)- and Cr(IV)-bearing species. Those few pentavalent chromium compounds that attain the longest lifespans are complexes formed with the oxochromium(V) ion CrO^{3+}. Structurally, unlike the other chromium species, most Cr(IV) and Cr(V) complexes more often assume tetrahedral arrangements than the compact octahedral form.

Most biologically encountered Cr(IV) and Cr(V) ions are formed as transient intermediates during the reduction of Cr(VI) to Cr(III). Although the Cr(V) species are somewhat more stable than Cr(IV), the magnitudes of both their half-lives amount to only minutes.[2,13] Due to these inherent instabilities, the Cr(IV) and Cr(V) ions are now considered likely to be responsible for the generation of cytotoxic and genotoxic radicals within cells.[1,17,18]

Hexavalent chromium (Cr[VI]) compounds nearly always exist as oxides or oxohalides. The ability for oxides to polymerize is a function of both the Cr–O bond character and the pH of the microenvironment. Unlike less acidic oxides (i.e., V[V], Mo[VI], W[VI]), Cr(VI) does not give rise to many polyacids/polyanions. This is likely the result of the greater extent of multiple bond formation between the Cr and O atoms.[10,11] In basic solutions (pH > 6), chromate ions (CrO_4^{2-}) predominate; between pH 2 and 6, the dichromate species ($Cr_2O_7^{2-}$) and $HCrO_4^-$ ions exist. Under extremely acidic conditions (pH < 1), H_2CrO_4 is predominant. The differing chromate species also display disparate oxidizing potentials. Acid solutions of dichromate are strong oxidants ($E^\circ = +1.35$ V), whereas the chromate ion is less oxidizing ($E^\circ = -0.13$ V). Dichromate ions, unlike any of the lower oxidation state species, also have the capacity to form peroxochromate compounds. However, these substances are extremely unstable and are dangerously explosive in the air.

It is chemically difficult to displace the oxygen atoms from the chromate ion, thus chromates are considered kinetically nonlabile. Conversely, the hydroxyl groups of chromic acid are more easily replaced to permit ester formation with hydroxyl- or thiol-bearing molecules (i.e., oxy acids, alcohols, thiols, and carboxylates). Because greater levels of chromic acid and ester formation occur primarily at lower pH values, their relevance under physiological conditions is small.[11,12]

III. ESSENTIALITY OF CHROMIUM COMPOUNDS

By definition, a nutrient is essential if a reduction in its total daily intake below some minimal level consistently induces signs of deficiency, and if subsequent resupplementation prevents and reverses the metabolic changes. Chromium has been deemed an essential micronutrient based on experimentally induced deficiencies in laboratory animals, and upon clinically observed changes in the health of children,[19,20] pregnant women,[21] and patients who are incapable of normal dietary intake.[22-24] The most commonly observed symptoms of chromium deficiency in humans include impaired glucose tolerance,[25,26] glycosuria, and elevations in serum insulin,[27,28] cholesterol,[29-34] and total triglycerides.[30] In animal models, in addition to the above-listed manifestations, there also is a decrease in longevity,[35] impaired growth,[36,37] altered immune function,[38] disturbances in aortic plaque incidence and size,[39,40] corneal lesion formation,[41] and an overall decrease in reproductive functions.[42]

In general, hexavalent chromium compounds are better absorbed through the intestinal mucosa than are the trivalent species.[43] However, due to the actions of stomach acid and other components within the gastrointestinal tract, most of an ingested Cr(VI) dosage is converted to Cr(III).[30] In this state, the uptake of the metal is low, about 1 to 3% of any given dose.[44,45] The presence of chelating agents or other stability-enhancing polypeptides/proteins greatly increases the ability of the ingested chromium to cross the jejunal mucosal membranes.[46]

The primary agent required for the proper uptake of dietary chromium is glucose tolerance factor (GTF).[25] Although low amounts of

GTF are synthesized in the liver and kidneys, GTF is most abundant in Brewer's (Torula) yeast.[47] GTF binds Cr(III) very efficiently in a Cr(III)-dinicotinic acid-glutathione complex, thereby ensuring enhanced uptake and preventing any redox interactions with other dietary or cellular components.[30,48] Numerous clinical[27–33,49–51] and animal[26,34,52–57] studies have demonstrated that the supplementation of GTF to standard diets or parenteral solutions results in the rapid reversal of many of the symptoms of Cr-deficiency. The efficacy of GTF as a chromium delivery agent is clear in that only 7 to 10 μg/day of Cr as Cr-GTF is needed to reverse glucose intolerance in adults. In comparison, 150 to 200 μg/day of free $CrCl_3$ is required to produce the same results.[58]

Not all studies reported have demonstrated a beneficial effect from chromium supplementation. The response itself appears to be related to the form and amount of the chromium supplied, the duration of delivery, and the chromium status of the test subjects. Some studies of diabetic patients reported low levels of improvement following chromium supplementation. While long-term chromium supplementation did eventually aid some patients, short-term treatments did not affect any of the parameters associated with glucose metabolism, primarily glucose tolerance and fasting/blood glucose levels.[27,49,59] Similar results demonstrating a lack of any beneficial effects from chromium supplementation have been obtained with several animal models (reviewed in Anderson, 1987).[55,60]

As indicated by the symptoms associated with chromium deficiency, it has been suggested that the essentiality of chromium is in part related to insulin function.[61,62] Chromium deficiency results in relative insulin resistance such that greater than normal levels of insulin are required. Observed changes include alterations in responsiveness of epididymal fat tissues,[61] decreased blood clearance of glucose,[26] and the modified cell transport of nonutilizable sugars such as D-galactose in Cr-deficient subjects in response to insulin; this suggests that chromium may be required to help mediate insulinic effects upon cell transport mechanisms.[62] Recent studies have suggested that chromium, either as a free form or in a loose association with the GTF carrier, forms a ternary complex with both insulin and its receptors.[63]

In addition to the effects of chromium deficiency upon insulin-related metabolic processes (i.e., glucose uptake and utilization, mobilization of free fatty acids, formation of low density lipoproteins),[31,64] several other physiological alterations have been shown. In studies of patients maintained on Cr-free total parenteral nutritional regimens, increased incidences of neuropathy, altered nitrogen balance, and even central encephalopathy were observed.[22–24] A role for chromium in the prevention of cardiovascular disease also has been demonstrated.[59,65] Epidemiological evidence suggests that low levels of dietary chromium are correlated with increased incidences of coronary artery disease.[66,67] Although the precise function of chromium in preventing coronary disease is unclear, its role in maintaining proper lipid, carbohydrate, and protein metabolism in the body through the action of insulin is the likely basis for this correlation.[60]

IV. CELLULAR UPTAKE OF CHROMIUM COMPOUNDS

It was shown in a number of studies that hexavalent chromium readily enters cells, whereas cells are relatively impermeable to trivalent chromium.[43] Trivalent chromium in the blood tends to bind with plasma proteins (such as transferrin) with little penetration into erythrocytes.[68,69] Even when bound in complexes with lipophilic organic ligands, e.g., glycine, 2,4-pentanedione, glutathione, and *o*-phenanthroline, the uptake of Cr(III) by red blood cells is very slow and three orders of magnitude lower than that for Cr(VI).[43]

The "uptake-reduction" model of Cr(VI) metabolism was originally proposed by Jennette.[70] According to this model, Cr(VI) crosses the cell membrane in the form of the tetrahedral chromate anions using the general anion transport system. This was based on the knowledge that a number of tetrahedral physiological anions, such as SO_4^{2-} and PO_4^{3-}, enter cells through a relatively nonselective anion channel. The penetration of Cr(VI) into erythrocytes is so rapid and nonspecific that radioactive chromium (^{51}Cr) has been extensively used in clinical studies as an erythrocyte tagging agent.[68] The effects of specific inhibitors suggest that Cr(VI) penetrates the erythrocyte membrane via the general anion

channel protein, referred to as the band 3 protein.[71] Two stilbene inhibitors, dinitrostilbene-2,2′-disulfonate[72] and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS),[73] which directly bind to the band 3 protein, greatly inhibit Cr(VI) uptake by bovine red blood cells *in vitro*.[74] Experiments with other cell types, including hepatocytes and thymocytes, indicate that the nonspecific anion channel is the overall common mechanism for Cr(VI) uptake in exposed hosts. Conversely, the kinetically inert Cr(III) ions, being predominantly octahedral, can cross the membranes only very slowly and by simple diffusion.

When either erythrocytes or pure hemoglobin are incubated with Cr(VI), the binding of the total available metal is 95 and 55%, respectively.[72] The binding of Cr(VI) to pure hemoglobin can be increased by the addition of reduced glutathione (GSH) to the reaction mixture. This suggests that, *in vivo*, intracellular GSH may be involved in the binding of chromium to red blood cell hemoglobin.[73] However, extracellular GSH significantly reduces the uptake of Cr(VI) by intact cells. This is likely the result of redox reactions mediated by the cysteinyl thiol group, which results in the reduction of Cr(VI) to Cr(III). Similarly, extracellular ascrobate also acts as a redox reactant to reduce Cr(VI) by intact cells.[75]

The uptake of Cr(VI) in human and rat erythrocytes *in vitro* follows a similar biphasic pattern. The initial phase has a half-time on the order of seconds, while the second phase is much slower, with an estimated half-time of approximately 10 min.[76] This second slower phase implies that the transport of chromate into cells is a saturable process. In addition to acting as a potential extracellular redox agent, GSH can function as a sink for intracellular chromium ions. The rapid complexation of Cr(VI) to GSH leads to a sequestration of free metal ions. This, in turn, causes a concentration gradient to form, thereby driving the further entry of chromium into the cells. A very rapid depletion of reduced intracellular thiols is noted only after a 2-h incubation of intact erythrocytes with Cr(VI).[77] In addition, preincubation of intact red blood cells with a GSH-depleting agent, like diethyl maleate, inhibits subsequent uptake of Cr(VI).[78] Conversely, the presence of dithiothreitol has been shown to enhance Cr(VI) uptake, most probably by its ability to maintain membranous and intracellular protein thiol groups in their reduced states.[78] Many of these results also have been reproduced in other cell types[79] and in intact hosts[80] with these and other GSH-depleting agents, such as buthionine sulfoximine.[81]

The impermeability of cells to Cr(III) is not absolute. Compounds containing Cr(III), especially those that are less water soluble, may be taken up by cells by endocytosis or pinocytosis. These processes have been interpreted to be the mechanisms responsible for the widely observed genotoxic effects of Cr(III) compounds in most prokaryotes and in some cultured eukaryotic cells that are phagocytically active.[82–84]

V. INTRACELLULAR AND EXTRACELLULAR REDUCTION OF CHROMIUM COMPOUNDS

As is noted later in this review, the hexavalent chromium compounds are the agents associated with the formation of cancers in exposed humans and experimental animals. Their strong oxidative chemical nature may be the underlying basis for their genotoxicity.[85–91] Oxidative DNA damage mechanisms have now been described for several metal carcinogens.[92–97] However, *in vitro* studies of purified DNA incubated with various Cr(VI) compounds have demonstrated that there is a very weak association between the chromium compounds and the negatively charged polynucleotide chains.[98–105] Many studies have in fact shown that the predominant form of chromium recovered in blood, tissues, and from cells cultured with Cr(VI) agents is trivalent.[16,80,106–112] Within cells, Cr(VI) is reduced to Cr(III), thereby generating unstable intermediate Cr(IV) and Cr(V) ions, active oxygen agents (HO·, singlet oxygen, and superoxide anion [$O_2^{\cdot -}$]), and thiyl and organic radicals (RS· and R·), all of which are ultimately responsible for the DNA damage observed.

Although the intracellular reduction of Cr(VI) is required for DNA damage, extracellular reduction decreases chromium entry into cells[75,77,79,113,114] and thereby serves as a host detoxification mechanism. Using both enzymatic

and chemical redox reactions, several agents that exist both within cells and as components of blood may act as reductants. Among these are reduced GSH,[3,4,18,78,89,115–117] lipoic acid,[18] glucose,[6] vitamins C (ascorbate),[7,17,18,118–120] E (α-tocopherol), and B_2 (riboflavin),[121–125] nicotinamide (NAD(P)H),[126] certain amino acids,[18,127–130] and hydrogen peroxide.[131,132] Other predominantly intracellular agents include microsomal cytochrome P_{450},[1,81,126,133–135] NADPH:quinone oxidoreductase (DT-diapharose),[136–138] several components of the electron transport chain,[8,139–142] and hemoglobin.[68,143]

Although Cr(III) does not enter cells very well,[43] the presence of cation-binding sites along the cell membrane does allow for Cr(III) accumulation.[144] Although the ramifications of this are not clear, competitive inhibition of the binding of other positively charged essential agents can serve as an indirect mechanism of toxicity. In addition, surface-associated Cr(III) compounds may be endocytized[82] and lead to genotoxic damage, as has been observed in phagocytes.[145]

The majority of Cr(VI) that enters the body via ingestion or inhalation is quickly reduced to Cr(III). Oral intake results in the rapid reduction to the poorly absorbed trivalent form by components of saliva and gastric juice. Any nonreduced Cr(VI) that remains is absorbed from the intestines and enters erythrocytes during transport in the portal vein.[87] Most of this Cr(VI) is then reduced to Cr(III) by intracellular reductants of the red (i.e., hemoglobin) or white (i.e., intraphagosomal action) blood cells, such that little of the original dose is still in the hexavalent form.

In a similar manner, inhaled Cr(VI) is acted upon by alveolar macrophages (in conjunction with other diverse cells throughout the lung) and epithelial-lining fluids within the bronchial tree. Their actions reduce the metal to its trivalent form and thereby greatly diminish the amount of Cr(VI) that might enter the bloodstream after crossing the alveoli.[146] The Cr(VI) that does enter the blood is reduced to Cr(III) by redox reactions with several blood-borne constituents and within red blood cells themselves, as is discussed later. The extracellular Cr(III) ions are then bound to the β-globulin portions of serum proteins, transferrin, albumin, or α_1- or α_2-globulin, for delivery to the kidneys and, ultimately, excretion from the host.[68,143,147]

Those Cr(VI) molecules that exist as oxyanions at physiological pH (i.e., CrO_4^{2-}) and are still able to reach intact cells may then cross over the cell membrane through the same relatively nonselective anion channels used by SO_4^{2-} and PO_4^{3-} anions.[43,73,75,77,79,111–114] The intracellular reduction to Cr(III) helps to create and maintain a concentration gradient for Cr(VI), so that under *in vitro* conditions, almost 100% uptake occurs.

Among the numerous intracellular reducing agents, those that are present in the highest concentrations are the small metal-reactive thiols, including GSH and cysteine. It has been demonstrated that, at physiological pH, GSH can reduce free Cr(VI) at significant rates.[18,115] The reactions give rise to thiolate-Cr(VI) complexes ($RSCrO_3^-$), which can react further to yield unstable Cr(IV) and Cr(V) intermediates and thiyl radicals.[3,129,130,148–150] Similar reactions with oxidized GSH or cystine can occur, but in these cases, without the available thiol group, only the primary amine and carboxylate moieties can interact directly with the metal.[151,152] Clearly, the normal role as a detoxification agent for GSH does not hold when confronted with intracellular Cr(VI). Several studies have directly implicated GSH in the ultimate mechanisms related to Cr(VI) genotoxicity. Treatment of cells with antioxidants (i.e., tocopherol), modulating the cellular levels of GSH with *N*-acetyl-L-cysteine, buthionine sulfoximine (BSO), or Na_2SeO_4 (sodium selenite), or pretreating cells with GSH directly all modified the amounts of genetic damage incurred by Cr(VI).[81,93,121,125,153] In those studies where intracellular levels of GSH were increased, there was a consistent corresponding increase in the total amounts of DNA strand-breaks or Cr-DNA adducts.

Like GSH, ascorbate exists in millimolar quantities in several tissues.[154] The reduction of Cr(VI) by ascorbate is more kinetically favored than the reduction catalyzed by GSH or cysteine.[18,118] Although GSH/cysteine follows a two-step pathway (i.e., overall second-order rate kinetics) for a two-electron reduction of Cr(VI) to Cr(VI),[18,148] ascorbate readily accepts the electrons in a unimolecular first-order reaction.[155] Unlike GSH, ascorbate does not rely on the avail-

ability of a single reduced thiol moiety for the redox reaction, but rather, it utilizes neighboring carbonyl groups for the ultimate generation of the stable diol-bearing dehydroascorbate molecule.

As an extracellular agent, ascorbate is well known to provide protection from the toxicities of chromate.[18,156–158] However, as with GSH, its participation in intracellular reduction is correlated with increased amounts of Cr-related genetic damage.[120] Increases in cellular levels of ascorbate have been found to lead to increased amounts of Cr(VI)-induced DNA-protein cross-links. However, at the same time, the numbers of induced alkali-labile sites, which may result from direct oxidative damage to DNA, were decreased by addition of ascorbate.

Among the many intracellular enzymes, it appears that those found in intact mitochondria have the most relevant and demonstrable impact on Cr(VI) reduction. Although <10% of any given dosage of hexavalent chromium is recovered from intact mitochondria,[110,159,160] oxygen consumption by the Cr-bearing organelles is greatly reduced.[140–142] Upon entering the mitochondria, Cr(VI) is reduced to Cr(V),[8,138,139] and the Cr(V) generated oxidizes NADH. This, coupled with the direct inhibition of α-ketoglutarate dehydrogenase by the Cr(VI) ions, effectively inhibits respiration[142] and decreases cellular levels of ATP and GTP.[141]

The components of the electron transport chain that might be participating in the reduction of Cr(VI) have been identified. In studies using substrates and known inhibitors of individual enzymes within the organelle, it was shown that mitochondrial electron transport chain complex I (NADH:ubiquinone oxidoreductase), II (succinate:ubiquinone oxidoreductase), and IV (ferrocytochrome c:oxygen oxidoreductase) are capable of reducing Cr(VI) to Cr(V).[8] Although the formation of the unstable penta- and tetravalent chromium species within the mitochondria may not present itself as a factor that directly contributes to cellular genotoxicity, the aberrant oxygen metabolism that does result may in fact cause altered concentrations of oxygen elsewhere in the cell. The greater presence of oxygen within the cytoplasm would lend itself to further interactions with nonmitochondrial Cr(VI) and the subsequent formation of activated oxygen species.

It has been suggested that other cellular enzymes, including microsomal cytochrome P_{450}, glutathione reductase, DT-diapharose, and aldehyde oxidase, may partake in the reduction of Cr(VI). However, under even very low oxygen conditions, the P_{450}-reducing activity is abolished.[134,135] For DT-diapharose, studies using the diapharose inhibitor dicumarol added to liver homogenates resulted in decreased Cr(VI) reduction.[137] In its purified form, diapharose has no reducing activity;[136] when combined with NADH/NADPH, however, a rapid two-electron reduction of Cr(VI) to Cr(IV) is thought to occur.[87] The physiological roles for aldehyde oxidase and glutathione reductase in the processing of Cr(VI) are not well defined. *In vitro*, both enzymes have been shown to interact with Cr(VI) ions. Aldehyde oxidase directly reduced Cr(VI) to Cr(III), but the mechanism of action is not clear.[161] Incubation of Cr(VI) with the reductase results in its inhibition, but the formation of Cr(III) has not been demonstrated. The direct oxidative effect of Cr(VI) on the essential cofactors NADH/NADPH may be an underlying basis for this reaction, but in studies with and without the cofactors, the results have been equivocal.[162,163]

It is apparent that the thiol-bearing substances and ascorbate are the likely regulators of the intracellular valence state for chromium. The redox reactions that occur, rather than being detoxifying, serve to promote the ultimate genotoxic activity of chromium. For successful therapeutic prevention of chromium genotoxicity, the protective agents must act at the point of cell entry or they must remain as extracellular constituents of the blood or other body fluids.

VI. GENOTOXICITY OF CHROMIUM COMPOUNDS

Chromium compounds are defined as genotoxic as a result of the genetic perturbations that have been observed *in vitro* and in bacterial and mammalian cells (reviewed in References 90, 104, and 164). At the DNA level, chromium compounds can produce DNA strand breaks,[81,93,123,124,165–168] DNA-DNA and DNA-protein cross-links,[87,93,98,104,123,166–175] and modified nucleotides, including oxidized base damage *in vitro*[176,177] and *in vivo*.[178] *In vitro* studies with

Cr(VI) and ØX174 bacteriophage DNA resulted in depurination of the DNA,[178-180] leaving residual alkali-labile abasic (AP) sites, which are potentially mutagenic.[181,182] At the cellular level, chromium compounds can yield specific locus mutagenesis (reviewed in the next section), induce the bacterial SOS repair/mutagenesis process,[183] induce λ phage,[184] and alter the expression of other inducible genes such as metallothionein and cytochrome P_{450}.[185,186] Although much of the genotoxicity data has been derived from studies of Cr(VI) compounds, other chromium oxidation states, including Cr(V), Cr(V)-complexes, or Cr(III), have been shown to produce genotoxic responses. Although the trivalent chromium compounds are not as active as the hexavalent compounds in cellular systems due to their poor uptake,[187] Cr(III) reacts *in vitro* with DNA. Trivalent Cr has been shown to bind to isolated nuclei *in vitro*,[102] to interact with nucleotides and nucleic acids,[187-189] to produce DNA-protein cross-links (reviewed in Reference 190), and to modify the fidelity and kinetics of DNA replication.[191,192]

In mammalian cells, even low levels of chromate are strongly clastogenic, producing frequent chromosomal aberrations such as chromatid gaps and breaks.[166,193-196] Chromium-generated chromosomal damage is randomly produced in the genome throughout the cell cycle,[193] although a slight S phase preference for DNA strand-break production has been noted.[167] Recently, lead chromate particles, which are among the least soluble chromium salts, have been shown to induce chromosomal damage in Chinese hamster ovary cells and human foreskin fibroblasts.[197] The antioxidant vitamin E has been shown to reduce the cellular levels of Cr(V) and to inhibit chromate-induced DNA strand-breaks and chromosomal aberrations in Chinese hamster V79 cells.[121,125,196] This suggests that reactive oxygen species may play a role in the genotoxicity of chromium compounds (reviewed in References 91, 96, and 97). Confounding evidence for the activity of oxidative processes in chromium genotoxicity has been suggested by other studies of oxidation mediators and radical scavengers on the inhibition of chromium-induced DNA strand-breaks.[93,116,132,198,199] The spin trapping of activated oxygen species produced by chromate and ascorbate reactions was recently reported.[119] Chromium compounds, including Cr(III) agents, also induce sister chromatid exchanges (SCE) *in vitro*,[82,86,87,144,166] and increased levels of SCEs have been noted among chromate workers.[200] Chromium-induced spindle disruption leads to chromosomal segregation difficulties, which result in aneuploidy and micronuclei formation.[90,201] Chromosomal aberrations also have been observed for several chromium compounds in non-mammalian species, such as yeast, plants, and insects (reviewed in References 90, 201, and 202).

VII. MUTATIONS INDUCED BY CHROMIUM COMPOUNDS

Chromium(VI) compounds are mutagenic as evidenced by data from several bacterial and mammalian mutagenesis studies (reviewed in References 86, 87, 90, 104, and 201). In *Escherichia coli*, base substitution mutations are detected following treatments with K_2CrO_4; however, these responses were elicited at very toxic doses yielding <10% survival.[166,203-205] In *Bacillus subtilis*, chromate and dichromate compounds, but not $CrCl_3$, were found to be mutagenic.[206] In *Salmonella typhimurium*, Cr(VI) compounds ($Na_2Cr_2O_7$, CrO_3, and K_2CrO_4) were mutagenic in several of the Ames strains.[207] Chromate primarily yielded base substitution mutations in the *his* locus of the *Salmonella* tester strains; however, some frameshift mutations were detected.[204,208] Base substitution mutagenesis is more frequent in the Ames strains that carry the pKM101 plasmid, and mutations predominated at A-T rather than G-C sequences.[209] Although Cr(VI) compounds were readily mutagenic in *Salmonella*, Cr(III) as $CrK(SO_4)_2$ and Cr(II) as $CrCl_2$ were not.[208] When complexed with organic ligands such as 2,2′-bipyridyl (bipy) or 1,10-phenanthroline (phen), however, some Cr(III) complexes exhibited mutagenic activity in *Salmonella* strains TA98, TA100, and TA92.[210] In the oxidation-sensitive *Salmonella* strains TA102 and TA2638, the 2,2′-bipyridyl Cr(III) complex was mutagenic only under aerobic conditions, whereas hexavalent potassium dichromate required oxygen for mutagenesis of TA102 but not TA2638 cells.[211] In a recent study of Cu(II) chromate and dichromate complexes with organic li-

gands (bipy) or (phen), only the $Cu(bipy)Cr_2O_7$ complex was highly mutagenic to TA102 cells; however, the mutagenic potency was lower than that for potassium chromate.[212] Although chromium compounds are quite toxic to yeast, cells resistant to the metal are readily recovered in industrial waste sites.[202,213] In *Saccharomyces cerevisiae*, chromate can induce viable *petit* mutants,[214] supporting other observations that mitochondria are subcellular targets of chromium genotoxicity.[142,215]

In mammalian mutagenesis assays, Cr(VI) compounds are often less mutagenic than in the bacterial assays, and yield mutants in most but not all assays. This may be related to the difficulties encountered in defining a suitable experimental dose range inasmuch as the chromium compounds are often effective over a very narrow dose range.[195,216,217] It also may be due in part to residual toxicity, which may result from reduced trivalent Cr that becomes trapped within the cells by its inability to escape through the cellular membrane.[18,70,111,114] In fact, residual chromium toxicity has been observed by the reduced plating efficiency of Chinese hamster V79 cells even 1 week after chromate treatment,[217] and may be reflected in the mutagenesis curves, which often plateau or decrease at the highest doses.[90,166,196,217–219] Vitamin E has been reported to reduce chromate cytotoxicity in Chinese hamster V79 cells,[125] further supporting a role for oxidative mechanisms in chromium genotoxicity in mammalian cells. However, chromium metabolism is complex, and ascorbic acid has been shown to increase Cr(VI) cytotoxicity and DNA-protein cross-links and decrease the formation of alkali-labile DNA damage,[120] possibly by increasing the intracellular levels of Cr(III).

For some mammalian genes, such as the X-linked hypoxanthine guanine phosphoribosyltransferase (*hprt*) locus in Chinese hamster cells V79 and CHO, the mutagenic response to chromate is moderate to strong, and varies for different chromium compounds.[195,219,220] With slightly soluble calcium chromate ($CaCrO_4$), 6-thioguanine resistance (*hprt*$^-$) is induced to high levels in Chinese hamster ovary cells[211] and human fibroblast cells,[222] whereas other more soluble chromate salts (sodium and potassium) were less mutagenic. In CHO cells, *hprt* mutagenesis could not be induced by insoluble lead chromate particles,[223] although these particles have recently been shown to be highly clastogenic.[197] However, chromium compound solubility is not the only determinant in chromium mutagenesis. In the mouse lymphoma L5178Y assay, for example, calcium chromate is relatively nonmutagenic at the non-X-linked thymidine kinase (*tk*) locus, whereas soluble chromate salts (K_2CrO_4 and $K_2Cr_2O_7$) are highly mutagenic in these lymphoma cells.[224] These compound-specific responses are opposite those observed for the *hprt* gene in Chinese hamster cells. In comparison to mutagenesis at the X-linked *hprt* gene, the mutagenic response of soluble chromate salts at the autosomal *tk* locus was much greater in the mouse lymphoma cells.[224] This may be related in part to the sensitivity of the *tk* locus toward the recovery of deletion mutations, which are often associated with clastogen mutagenesis.[225]

The mammalian mutagenesis data suggest that chromium mutagenesis can be influenced not only by the nature of the chromium compound and the experimental cell line studied, but also can vary for different genetic loci. Chromium compounds are not mutagenic to all mammalian loci. Although low levels of chromium-induced mutagenesis were observed for the essential *Na^+K^+/ATPase* gene in Chinese hamster ovary cells,[221] almost no response was detected at the same gene in Chinese hamster V79 cells.[217,218] Mutagenesis at this locus, however, is generally restricted to a limited subset of base substitution mutations,[226] and the gene is not believed to tolerate deletions. Even at the clastogen- and deletion-sensitive *gpt*$^+$ locus in transgenic V79-derived cell lines,[227,228] potassium chromate and dichromate yielded only moderate mutagenesis, although preliminary data suggested recovery of a significant number of deletion mutations.[340]

In addition to inhibiting chromate cytotoxicity in mammalian cells, vitamin E can inhibit the mutagenicity of sodium chromate at the *hprt* locus in Chinese hamster V79 cells.[196] However, the metabolism of chromium compounds is quite complex, as already discussed, yielding unexpected results. At high doses (200 μM), vitamin B_2 was shown to reduce chromium cytotoxicity in Chinese hamster V79 cells;[123] however, more recent studies showed that much lower doses of riboflavin could potentiate the induction of Cr(VI) mutations and chromosomal aberrations with no

effect on cytotoxicity.[216] Until recently, it was not known what specific mutations could be produced in mammalian genomes by chromium compounds. A recent investigation of DNA sequence alterations induced by chromium(VI) oxide in *hprt*$^-$ mutants of Chinese hamster ovary cells showed a predominance of mutations in A-T-rich gene sequences, which yielded frequent T→A and T→G transversions.[229] Although many of these mutations were single base substitutions, insertions, or deletions, a significant proportion (~20%) of these mutants harbored substitutions at two adjacent bases, and one mutant showed four base changes. Additionally, up to 20% of the chromium-induced *hprt* mutations may involve deletions because not all mutant DNA allowed PCR recovery of the *hprt* sequence. A similar mutagenic spectrum also was observed for mutants induced by potassium chromate and lead chromate in these cells,[229] and this spectrum differs significantly from that found in spontaneous *hprt*$^-$ mutants. The specificity for chromium-induced base substitution mutagenesis of A-T- rather than G-C-rich DNA sequences in mammalian cells correlates well with earlier *Salmonella* data; however, the particular reactive intermediates that produce these alterations have not yet been defined. It is essential to determine whether chromium-induced mutagenesis or carcinogenesis is due to the direct action of chromium complexed to cellular ligands, or to intermediates such as activated oxygen species that may be produced during the intracellular reduction of Cr(VI).

VIII. CHROMIUM CARCINOGENICITY IN ANIMAL MODELS

Many of the earliest attempts to determine which species of chromium compounds were the causative agents for occupationally related cancers utilized inhalation or parenteral exposures with metallic chromium, chromite ore, or several commonly utilized chromium compounds.[230–234] Although the majority of these studies yielded negative or equivocal results, several studies showed that the Cr(VI) species were often carcinogenic in test animals.[235–240]

Oral exposure to chromium compounds does not result in enhanced tumor formation in test animals when compared with vehicle controls. Rats and mice provided with chromic acetate in their drinking water for life did not develop tumors at various body sites at greater rates than did controls.[240,241] When Cr_2O_3 (1800 to 2850 mg/kg/day) was ingested along with other solids (i.e., baked in bread), there again was no enhanced incidence of tumors.[242] Similar long-term feeding studies with various Cr(VI) compounds are still lacking, although one study indicated that while the total incidence of cancers did not vary from the controls, the development of forestomach carcinomas (as opposed to forestomach papillomas only) occurred only in rodents fed K_2CrO_4 in their drinking water at 9 mg/kg/day for 900 days.

Although the corresponding linkage or route of exposure and sites of tumor formation are remarkably similar between humans and mice/rats, this susceptibility to tumor formation varies widely among the commonly used animal models. For example, mice and rats exposed to $ZnCrO_4$, $CaCrO_4$, or $Na_2Cr_2O_7$ atmospheres or intratracheal implants displayed a greater incidence of lung squamous metaplasias, subsequently followed by lung adenomas or adenocarcinomas, than did controls.[243–247] Conversely, rabbits, hamsters, or guinea pigs exposed to these agents failed to develop lung tumors.[248–250]

By using more invasive means of exposure, cancers could be induced in mice and rats following implantation of less soluble chromate compounds primarily. In no cases, using intratracheal, -bronchial, -muscular, -peritoneal, -venous, or -femoral implantation, did metallic chromium or trivalent chromium compounds give rise to increased incidences of tumors in the hosts.[231,234,240,242,244,251–258] Subcutaneous implantation of aqueous suspensions or gelatin capsules containing large doses (>10 mg) of strontium, calcium, or lead chromates (yet, oddly, not barium chromate) resulted in spindle-cell sarcoma, rhabdomyosarcoma, or fibrosarcoma formation, but only at the injection site.[233,259–261] Intramuscular injections of several forms of these poorly soluble chromates in sheep fat, arachis oil, or trioctanoin vehicles also caused tumor formation.[233,234,256,262,263] As was the case with all the observed tumors formed after chromate implantation, none of the tumors were apparently metastatic.[263] Similar implantation studies with highly

water-soluble $Na_2Cr_2O_7$ or Na_2CrO_4 failed to demonstrate a similar rise in formation of in-site tumors.[234,243,256]

Direct inhalation and intratracheal, intrapleural, or intrabronchial instillation of Cr(VI) compounds are by far the most common routes of exposure, and tumor formation in animal models most often occurs at these sites of deposition. The preponderance of data indicates that neither metallic nor trivalent chromium compounds give rise to lung tumors. A single study using rats instilled with chromic(III) oxide reported increases in lung sarcoma formation;[264] however, the control group was not reported and so conclusions regarding carcinogenicity were difficult to establish.[235,236]

Using direct inhalation studies, mice chronically exposed to $CaCrO_4$ dusts or to chromic acid mists developed lung adenomas and carcinomas,[246,265-267] although the incidences were not statistically significant. Similar results were obtained with rats exposed to $Na_2Cr_2O_7$.[247] In studies of weekly intratracheal instillations of chromium compounds, both mice and rats developed numerous lung tumors. In mice, installation of basic zinc chromate resulted in the formation of benign adenomas, but at a rate no greater than that observed in vehicle controls.[248,250] With rats, too, installation of $CaCrO_4$ resulted in a greater formation of benign adenomas than adenocarcinomas, but this incidence was significantly higher than that observed in controls;[17] similar results were obtained with soluble $Na_2Cr_2O_7$. The interesting observation in this particular study was that instillation of an equivalent dose of either compound, when delivered either in a single bolus or cumulatively over five separate applications, led to dissimilar results. With the insoluble $CaCrO_4$, the single exposure caused an 18% incidence of adenomas/adenocarcinomas, twice that obtained in rats given five treatments. With the dichromate, only the single treatment induced tumor formation. Another intratracheal study indicated that, although lung cancer incidence was not increased by exposure to chromate dusts, the age at which tumors first appeared was lessened.[248,268]

To better examine the carcinogenic process in the lungs, an intrabronchial method of implantation was developed. The chromium compound of interest was placed in a metal basket or pellet and surgically implanted in the animal bronchus. By slow leaching, a selected zone of bronchial epithelium was subjected to exposure for a continuous period of time. Using this technique, it was demonstrated that many test compounds, including several trivalent species, gave rise to squamous metaplasias. However, only hexavalent agents caused significant increases in the incidences of metaplasia and subsequently carcinomas.[243,244,254] As in the subcutaneous implantation studies, barium chromate (and in this case, lead chromate) was not effective. When this protocol was used for introducing condensed welding fumes and thermal spraying fumes, a greater incidence of distal benign cancers (i.e., lymphomas, skin, intestine, central nervous system, thyroid, and pituitary) was obtained; yet no lung cancers were obtained.[269]

In summarizing the available data from all the animal studies performed, the IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans concluded that there was sufficient evidence for the carcinogenicity of soluble calcium chromate and several relatively insoluble hexavalent chromium compounds in laboratory rodents. The evidence is more limited for the carcinogenicity of chromic acid, cobalt chromium alloy, lead chromate oxides, or sodium dichromate in these animals. The data for the evaluation of the carcinogenicity of other hexavalent chromium compounds, metallic chromium, and the majority of trivalent chromium agents remain inadequate to date.[235,236]

IX. EPIDEMIOLOGICAL STUDIES OF HUMAN CANCER

Since the time when the first case of cancer in a "chrome worker" was reported,[270] a great number of epidemiological studies have been performed. In addition, a large number of case reports relating worker illness with chromate exposure have been published. The greatest levels of exposures to Cr(VI) occur primarily during chromate production, welding processes, chrome pigment manufacture, chrome plating, and spray painting. Exposures to other valence forms of chromium occur primarily during mining, ferrochromium and steel production, and during the cutting and grinding of chromium alloys.

In some of the epidemiological studies, the effect of chromate was evaluated in upward of several thousand workers exposed to chromium over widely ranging periods of time (a few months to 30 years). Although the vast majority of these studies deals with workers in the chromate production industry, there are fewer studies investigating the health effects in chromate pigment production workers and fewer yet examining workers in the chromium-plating and/or ferrochromium industries. The results of the major epidemiological studies of cancer formation in workers exposed to chromium during chromate production,[271-286] production of chromium pigment,[287-297] electroplating,[298-306] the production of ferrochromium alloys,[307-310] and other incidental industrial[311-317] and environmental exposures[318-321] are summarized in Table 2.

As noted, the most extensive epidemiological results have been obtained from workers in the chromate production industry. An increased risk for development of respiratory cancers following exposure to chromium particles and dusts in this industrial setting has been firmly established in epidemiological studies carried out in the U.S., U.K., Japan, Germany, and Italy. In other international studies, the risk of lung cancer development among workers involved in the production of chromium-containing pigments was found to be further augmented in those facilities where $ZnCrO_4$ was produced; no similar increased risk was noted in those workers producing $PbCrO_4$-containing pigment. In studies of workers involved in chrome plating, a significantly greater incidence of lung cancer in these workers also was apparent. In studies analyzing the incidence of lung cancer formation in ferrochromium plant workers, a weaker correlation has been established. One reason for this latter observation may be that, unlike in the other cited occupations, the predominant exposure in this industry is to Cr(III) compounds and metallic chromium, instead of more directly carcinogenic Cr(VI) agents. Finally, the results of studies on stainless-steel welders are consistent with the findings of increased mortality from lung cancers in other chromium-exposed workers. However, these studies by themselves do not independently contribute to the overall evaluation of the carcinogenic effects of chromium because welders are exposed to many other potential genotoxic/immunotoxic agents during their job performance.

In addition to the increased incidence of lung cancer among chromium workers, occupational exposure to chromates has been associated with high chromium concentrations in lung tissue and in hilar lymph nodes.[322-324] The chromium concentration in lung tissue also serves as a major criterion in establishing the causal connection between occupational exposures to certain chromium compounds and development of bronchial carcinoma.[325-327] The chromium content of the tumor tissue itself is not a useful criterion in this respect inasmuch as it is not known whether or not the tumors store the metal during their progression. The lung chromium content in workers who died of lung cancer after >10 years of employment in chromium product manufacturing plants was significantly higher than that in control nonexposed groups.[328] In one case, the chromium content in the lung tissues of a chromate worker involved in chromium production for 35 years, and who died from lung cancer, was 90 times the amount found in normal lungs.[329]

The chromium content of lung generally increases in direct relation to the duration of exposure. Most often, the concentration of chromium in the upper lobes is significantly higher than that in the lower lobes, suggesting regional differences either in clearance from, or deposition in, the lung. It has become apparent that the inhaled metal can remain in the lungs long after exposure to chromate had ceased. Although the chromium content in the respiratory system of chromate workers was higher than that in lungs of control groups, the amount of chromium found in nonrespiratory tissues was not significantly higher than in similar tissues obtained from controls.[330] The high lung chromium content did not have a direct relation to the occurrence of lung cancer, however, because the primary site of chromium-related lung cancer was the large bronchi and not the peripheral lungs, which contained the highest chromium content.

Several studies have addressed the problem of histopathological classification of cancers in chromium workers. Histopathological results differ depending on the type of chromium compound, the duration of exposure, and whether or not the workers were active smokers. Hueper[331] reviewed 123 cases of lung cancer and found 46

TABLE 2
Epidemiological Studies of Chromium-Induced Cancers

Exposed group	Site of cancer formation	Ref.
Chromate-producing industries		
U.S.	Respiratory system, digestive system, oral cavity	271–279
U.K.	Lungs, all other sites, nasal cavity	280, 281
Germany	Lungs, stomach	282
Japan	Respiratory system, stomach	283–285
Italy	Lung, larynx, pleura	286
Chromate pigment ($ZnCrO_4$ and/or $PbCrO_4$) production		
Norway	Lung, digestive system, nasal cavity	287, 288
U.K.	Lungs	289–293
France	Lungs	294
Germany and Holland	Lungs	295, 296
U.S.	Lungs, stomach	297
Chrome-plating industries		
U.K.	Lungs, nasal cavity, digestive system	298–300, 306
Japan	Lungs	301–303
U.S.		
Diecasting and Ni/Cr plant	Lungs	304
Italy (hard and bright)	Lungs, nasal cavity	305
Ferrochromium industries		
Soviet Union	Lung, all sites, esophagus	307
Sweden	Lungs	308
Norway		
Ferrochromium plant	Lungs, kidney, prostate	309
Ferrosilicon plant	Stomach	310
Incidental Cr exposure		
Japanese workers handling Cr	Lungs	311
U.S. aircraft production (spray painting/electroplating)	Respiratory system	312
U.S. Ni/Cr foundries	Lungs	313
Icelandic masons	Respiratory system	314
Danish/Swedish/Finnish hospital-based case control	Nasal and paranasal sinuses (odds ratio, 2.7)	315, 316
German extended case control	Bladder (odds ratio, 2.2)	317
Environmental exposure		
Swedish FeCr smelters	Lung	318
New Jersey chromite ore sites	Lung	319, 320
German Ruhr district	Lung	321

cases of squamous cell carcinoma, 66 cases of anaplastic-type cancer, and 11 cases with adenocarcinoma. In a separate study using cytological examinations performed on a group of 116 chromium-exposed workers, the incidence of abnormal cells (class III-IV Papanicolaou) was about 25% as compared to 6% in the polyvinylchloride industry, 1.6% in the general chemical industry, and 0.6% in heavy smokers.[332] A histopathological analysis of changes in the bronchial epithelium among chromate workers was performed in order to clarify the effects of chromate compounds.[333] Of 235 cross sections obtained from a small group of workers (n = 14), basal cell hyperplasia of the bronchial epithelium was found in 13%, squamous metaplasias were observed in 29%, and atypical metaplastic changes were noted in 34% of the samples. It was concluded that

inhalation of chromate dust affected the bronchial epithelium and caused highly atypical squamous metaplasias that subsequently developed into carcinoma *in situ*, and ultimately into invasive cancer. Thus, in a high-risk group such as chromate workers, emphasis on the early detection of lung cancer by serial sputum cytology, chest X-rays, and bronchoscopy is warranted.[334]

The type of cancer that develops following chromium exposure has been shown to vary with the chromium type and duration of exposure. In studies where the incidence of chromium-related lung cancer was shown to be 16 times higher in exposed workers than in the general population, the predominant cancers were squamous cell and small cell carcinomas.[330,335] Those patients who presented with small cell carcinomas were found to have been primarily engaged in the second phase of chromate production during which they were heavily exposed to Cr(VI) dusts. The patients who primarily developed squamous cell carcinomas were found to have worked in the second, third, and fourth stages of production, all of which involve exposures to increasingly refined Cr(VI) products and increasingly lower levels of Cr(III)-bearing dusts.

With regard to the duration of employment as a contributing factor to cancer development, in those chromium workers suffering from small cell carcinoma, the length of employment was significantly shorter that for those workers with squamous cell cancer. It follows then that as a function of total exposure, when the exposure to chromium agents was heavy, the major type of lung cancer that evolved was the small cell type.

An analysis of the correlation between cancer incidence and one of several other factors, such as duration of employment in the chromium industry, plant safety modifications, worker age at the beginning of employment, and estimations of the degree of chromate exposure, found that the duration of employment was the major dependent factor.[281] A positive correlation between the duration of exposure and lung cancer death was found in most of the studies.[274,283,292,293,297,304,312] Several studies also have shown that modifications in the plant and work environment have been associated with appreciable reductions in the overall increased risk from chromium-related lung cancer.[281,336] A significant downward trend in the reported numbers of bronchogenic carcinomas was found to coincide with major improvements in work conditions.[336] In fact, there has been no significant increase in lung cancer deaths among workers who started to work in plants after the completion of processing modifications.[293]

The possibility of exposure to chromium as a causative agent of cancers other than those in the respiratory tract also has been examined. In a study of five cases of cancer observed in a small group of chromium workers, a relationship between exposure to chromates and cancers in the gastrointestinal tract was suggested[337,338] An increase in the number of cancers of the digestive tract also has been reported in chromium electroplaters.[298,299] Långard and Norseth,[288] in a follow-up of the previously reported cohort study performed in a pigment-producing plant, found 3 cases of gastrointestinal cancer in a small subpopulation of workers (n = 24) with more than 3 years of employment. An increased risk of gastrointestinal tract carcinomas also was detected in workers employed in ferrochromium plants.[309] In one survey, 5 cases of gastric cancer were reported among 325 ferrochromium workers; this compared with an incidence of only 3 predicted for a control population of equal size.

A greater frequency of cancers of the upper respiratory tract and oral cavity also has been reported in chromate-producing industry employees. The cancers most often involve the buccal cavity, pharynx, and esophagus. An analysis of the occurrence of nasal and sinonasal cancers with respect to various occupational exposures has shown a positive association between these cancers and exposure to chromium in welding fumes, flame-cutter torch and soldering iron fumes, and chromium contaminants in hardwood, mixed-wood, and softwood dusts.[315,316] An increased risk for development of the rare sinonasal cancer was reported in epidemiological studies of workers employed in primary chromium production,[271,275,281,283] chromium pigment production,[287,288] and in chromium platers,[306] as well as in some hospital-based case control studies.[315,316] For cancers other than those of the lungs and sinonasal cavity, no consistent pattern of cancer risk has been demonstrated in those workers exposed to chromium.

Nonoccupational sources of chromium exposure include food, air, and water; however, the

levels of metal are several orders of magnitude lower than those encountered in occupational situations. Epidemiological studies have been performed examining the relationship between environmental exposure to chromium and mortality from lung cancer. In a population living near two Swedish ferrochromium smelters, lung cancer mortality was not different from the general national rate.[318] In Northern New Jersey regions surrounded by nearly 40 commercial and industrial properties with identified chromite ore-processing residues in the soil, two residential populations were examined. No significant increases in either noncarcinogenic or carcinogenic health hazards were observed, regardless of acute or chronic exposure to the tainted soils.[319,320] In these residential areas, the most important route of population exposure to chromium was through incidental soil ingestion. In the New Jersey studies, the cancer risk due to inhalation of suspended particles was estimated to be less than one in a million provided that the soil levels of Cr(VI) were <180 ppm. Conversely, high chromium and nickel emissions measured in the polluted environment around Bochum in the Ruhr region of Germany were associated with very high incidences and mortality from lung cancer.[321,339] At autopsy, the Bochum subjects presented lung concentrations of chromium and nickel that were fivefold higher than those in a comparison group from the less-polluted Munster region.

The IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans has summarized the data from these varied case reports, as well as data from epidemiological studies of industrial exposure to chromium in the chromate and chromate-pigment-producing, chromium-plating, and ferrochromium industries. They concluded that "there is sufficient evidence of respiratory carcinogenicity in workers occupationally-exposed during chromate production, chromate pigment production, and chromium plating. Data on lung cancer risk in other chromium-associated occupations and for cancer at other sites are insufficient. The epidemiological data do not allow an evaluation of the relative contributions to carcinogenic risk of metallic chromium, trivalent chromium, and hexavalent chromium, or of soluble vs. insoluble chromium compounds."[236]

X. CONCLUSIONS

This review was undertaken to provide the readers with a comprehensive overview of several aspects of the toxicology of chromium metal and its derivative compounds. As indicated throughout the text, chromium in its varied inorganic/organic forms has some very interesting properties, especially in biological systems. Under physiological conditions, hexavalent chromium exists as an oxyanion that is readily transported into cells via preexisting anion transport mechanisms. After entering the cell, Cr(VI) is readily reduced to intermediate Cr(V) and Cr(IV) states before finally attaining its ultimate form, Cr(III). If Cr(VI) is reduced to Cr(III) extracellularly, this form of the metal is not readily transported into cells and so toxicity is not observed. The balance that exists between extracellular Cr(VI) and intracellular Cr(III) is what ultimately dictates the amounts and rates at which Cr(VI) can enter cells and impart its toxic effects. Oddly, while Cr(VI) is regarded as the most toxic form of chromium, Cr(III) is essential for life. It is this interesting chemistry of the various valence states of chromium that makes this metal a complex agent in most biological systems.

The dynamic intracellular interactions of the redox-sensitive chromium species are ultimately the basis for most of the toxic effects manifested by cultured cells or viable hosts after chromium exposure. As genotoxins, Cr(VI) agents are positive in more genotoxicity assays than any other known carcinogen/mutagen. Their ability to induce many different types of genetic lesions is well-documented and was reviewed extensively here. In addition, a discussion of the mechanisms of mutagenicity/genotoxicity of the various chromium compounds was provided to help clarify why certain lesions are induced.

This review concluded with an overview of a critically important topic, chromium carcinogenesis, which is the predominant toxicologic effect of chromium compounds. Extensive evidence from both animal and humans studies was presented to provide the readers with a comprehensive up-to-date compendium that builds on those published earlier by several IARC Working Groups. The incidence and types of cancers formed in test animals and exposed humans were

reviewed here in the context of the chemistry of the particular chromium compound studied. From this, a clearer understanding of the role of metal valence, as well as compound speciation, was obtained from the numerous reports documented over the past century. This review did not address chromium toxicology in humans beyond the carcinogenic effects because the latter are the most significant toxicologic endpoints.

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