

Toxicity versus essentiality of chromium¹

by Jan Alexander, MD²

Chromium is recognized as an essential trace element for humans. Even though it has not yet been assigned a recommended dietary allowance (RDA) by the United States National Research Council (1), an estimated safe and adequate daily intake of 50–200 µg for adults has been set. Although it is known that chromium plays a role in glucose and lipid metabolism, the mechanisms of action and functional molecular role of chromium in intermediary metabolism in humans are still unclear. Chromium is important for glucose tolerance, and chromium-responsive insulin resistance has been reported. Substantial differences in biological activity between chromium compounds *in vitro* and *in vivo* have been demonstrated. Chromium deficiency can apparently be remedied by means of inorganic trivalent chromium compounds, as well as by the more readily bioavailable organic-bound chromium. The average absorbability of inorganic trivalent chromium present in the diet has been estimated to be about 0.5% (1).

Almost all of the sources of chromium in the earth's crust are in the trivalent state, and naturally occurring chromium compounds in the hexavalent oxidation state are rare. Hexavalent chromium compounds are thus man-made products (2).

Chromium toxicity is primarily associated with exposure to hexavalent chromium compounds. Acute and chronic toxicity at high doses, as well as immunologically dependent skin hypersensitivity and genotoxic and carcinogenic effects are well known (2–5).

Trivalent and hexavalent chromium compounds behave differently in the body. Hexavalent chromium can be reduced to trivalent chromium in extracellular fluids in the lung or gastrointestinal system. Whereas trivalent chromium compounds do not or only very slowly (1000-fold less than hexavalent chromium) penetrate (by endocytosis?) cells, hexavalent chromium is easily taken up by cells by the anion carrier in the cell membrane, and it is metabolized and trapped inside (3, 4).

A variety of enzymes and low molecular-weight substances present in the cell reduce hexavalent chromium. At physiological pH, the reduction processes appear to be kinetically rather than thermodynamically controlled (5). Consequently, only those reductants which react with hexavalent chromium at a significant rate are likely to contribute substantially to hexavalent chromium reduction in the cell. It follows that ascorbate, in particular, but also physiological thiol-containing reductants, such as glutathione (GSH) and cysteine, are the likely nonenzymatic biological reductants (3–5). Among enzyme proteins, endoplasmic P450, and particularly the ethanol-inducible form P450IIE1, have been shown to reduce hexavalent chromium *in vitro*, but this reaction is inhibited even by low levels of oxygen (6). Hexavalent chromium can also penetrate the mitochondrial membrane and be reduced (7).

Although hexavalent chromium can react at several places in the cell, the toxic effects seem to be fairly specific. The dramatic depression of mitochondrial oxygen consumption is apparently due to an inhibitory action of hexavalent chromium on α -ketoglutarate dehydrogenase, which supplies the respiratory chain with nicotinamide dinucleotide dehydrogenase (8).

Biological reduction reactions only take place in stages with one or two reducing equivalents. Hence, the reactive chromium species pentavalent and tetravalent chromium, but also hexavalent chromium esters, thiyl radicals, and reactive oxygen species, are formed during the biological reduction of hexavalent chromium (5). Further reduction of reactive chromium species, as well as the interaction of other reactive species with critical targets in the cell such as deoxyribonucleic acid (DNA), is assumed to be the basis for the toxic and genotoxic effects (3–5, 8). The end-product of the reduction process is trivalent chromium.

A variety of DNA lesions can be observed after hexavalent chromium exposure (3–5), for example, DNA strand breaks, DNA interstrand cross-links, DNA protein cross-links, and lesions caused by reactive oxygen species such as 8-OH-guanine formation. The binding of chromium and GSH to DNA has also been reported. Of these lesions, trivalent chromium can produce DNA protein cross-links in isolated nuclei, but otherwise, with few exceptions, it is *not* genotoxic in cellular assay systems, perhaps

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because of its inability to penetrate the cellular membrane. Thus DNA strand breaks and mutagenicity have been observed in cells in culture or bacteria treated with organic-bound trivalent chromium capable of penetrating the cell. Furthermore, the transfection of single-stranded bacteriophage DNA treated with trivalent chromium into *Escherichia coli* showed that trivalent chromium altered the interaction between the template and polymerase and resulted in reduced fidelity of DNA replication (9). The major lesions induced by hexavalent chromium in vivo appear to be DNA interstrand cross-links, DNA protein cross-links, and chromium-DNA adducts. These lesions are probably due to differences in repair kinetics. In vivo trivalent chromium treatment does not result in such lesions, but chromium binds to the nonhistone component of chromatin (4).

Trivalent chromium forms stable coordination complexes which have a very low exchange rate with biological ligands (4). However, the formation of labile pentavalent and tetravalent chromium species during reduction may facilitate ligand attachment and binding to critical target macromolecules in the vicinity of centers catalyzing hexavalent chromium reduction (3, 6–8).

Hexavalent chromium reduction in extracellular fluid should be considered a detoxification mechanism (3). Inside the cell, hexavalent chromium reduction constitutes a mechanism of both detoxification and activation, as a number of reactive species are formed. The site of reduction may be important, but some of the species formed are sufficiently long-lived (eg, in the form of coordinated complexes) to migrate from the cytoplasm of the cell into the nucleus and damage DNA (4, 5).

Since low-dose exposure to chromium compounds, particularly from food, is usually associated with low general toxicity (1), the main adverse effect to be evaluated in relation to essentiality is the carcinogenic effect. Thus the following two key questions are important for the evaluation of the risk associated with chromium exposure:

1. Is there a threshold for chromium genotoxicity and carcinogenicity? For the species ultimately damaging DNA and responsible for the genotoxic event, no threshold probably exists. If there is no threshold, is it limited to specific chromium compounds, eg, those of hexavalent chromium?

2. Does trivalent chromium play any role in the carcinogenic process?

Carcinogenicity has not been observed after oral exposure to trivalent chromium compounds (10, 11), nor are these compounds oxidized to hexavalent chromium. All chromium compounds entering cells are eventually converted into trivalent chromium and, since EDTA (ethylenediaminetetraacetic acid), a trivalent chromium chelating agent, disrupts DNA-pro-

tein cross-links, it can be inferred that these cross-links are mediated by trivalent chromium (12). It is, however, not known which of the genotoxic events are critical for the carcinogenic effect of hexavalent chromium compounds and whether intracellular trivalent chromium poses any threat to DNA in this respect. Cells seem to be well protected against extracellular trivalent chromium.

The carcinogenic risk associated with trivalent chromium exposure, if there is any risk at all, is probably of the threshold type (due to the kinetics) and very small. Exceptions cannot be excluded for organic-bound trivalent chromium compounds. The International Agency for Research on Cancer concluded in 1989 that trivalent chromium compounds are not classifiable as to their carcinogenicity to humans (11).

To clarify these problems, knowledge of the carcinogenic mechanisms of chromium is important, but equally important is an understanding of how chromium functions as an essential element and what role it plays in cellular and molecular metabolism in the human or animal organism.

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