21 Mode of Action and Toxicity of Trace Elements

ARUN K. SHANKER

Central Research Institute for Dryland Agriculture (CRIDA), Indian Council of Agricultural Research (ICAR), Santoshnagar, Hyderabad, 500 059, India

1 INTRODUCTION

Trace elements have made their entry into plant and animal systems due to their distinct chemical properties such as reduction and oxidation reactions under physiological conditions. On the other hand, the very same chemical reactions that make some of these trace metal ions obligatory for life are also the primary cause of their toxicity when present in surfeit. In analytical chemistry, a trace element is an element in a sample that has an average concentration of less than 100 parts per million (ppm) atoms, or less than 100 micrograms per gram. In biochemistry, a trace element is a chemical element that is needed in minute quantities for the proper growth, development, and physiology of the organism. In biochemistry, a trace element sometimes is also referred to as a micronutrient. In geochemistry, a trace element is a chemical element whose concentration is less than 1000 ppm or 0.1% of a parent rock's composition. The major difference between metals and other toxic substances and xenobiotics are that they are not created by humans, although several anthropomorphic activities contribute to the prevalence of these metals in proximity of human and plant ecosphere which facilitates their entry into the biological systems [Beijer and Jernelov, 1986]. The alteration of elemental stoichiometry by human activity is also another important way by which the trace elements acquire toxic properties. Historically, *The Ebers papyrus* (1500 B.C.E.) contains information pertaining to inquiries into the poisonous nature of metals such as lead, copper, and antimony. Metal toxicity and cure is documented from as early as 370 B.C.E. when Hippocrates described abdominal colic in a man who worked with metal extraction. From then on, there are several other references of studies in

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toxic interaction of metals—mainly lead, arsenic, and mercury—in humans. It must be emphasized here that most of the concerns until the mid-20th century among scientists were mainly on the acute and visible clinical effects mostly confined to humans.

The landmark inquiry into mechanism of action of heavy metals was the work by Voegtlin et al. [1923], wherein they reported the mechanism of action of arsenic on protoplasm. Diagnosis and treatment at a clinical level was more prevalent than inquiries into the mode of action and toxicity of trace elements. Later the logical evolution of the science moved toward the more subtle, chronic, or long-term effects of numerous trace elements which paved the way for the modern science of trace element toxicology at cellular and molecular levels. If one traces the history of the toxicology of metals over the past half-century, the role of the Pharmacology Department of the University of Chicago is paramount. The story commences with the use of uranium for the "bomb" and continues today with research on the role of metals in their relations with DNA, RNA, and growth factors. In fact, the Manhattan Project created a productive atmosphere that resulted in the commencement of quantitative biology, radiotracer technology, and inhalation toxicology. These innovations have revolutionized modern biology, chemistry, therapeutics, and toxicology. The behavior of dissolved metals has been studied for over 25 years. In the early 1970s, much research concerned the influence of pH and water hardness on metal toxicity to algae and other aquatic organisms. This work led to the development of a model to predict metal toxicity based on pH and water hardness [US EPA, 1986]. Currently, more interest is shown in trace element interaction with plants, microorganism, and animals due to the established link of theses biological systems with human life.

The list of trace elements essential for both animals and plants is large: It comprises vanadium to zinc in the first row series of the periodic table, plus molybdenum in the second row series. The physiological range for essentiality of these elements between deficiency, sufficiency, and toxicity is consequently exceedingly narrow, and there exists a controlled metal homeostasis network to adjust to fluctuations starting from nonavailability to toxicity [Beijer and Jernelov, 1986]. At a larger scale the effects of trace elements on the ecosphere is very detrimental as proved by the classical study of Watson et al. [1976], wherein emissions of heavy metals from a lead ore-processing complex caused perturbations to the litter–arthropod food chain in a forest ecosystem. Elevated concentrations of lead (Pb), zinc (Zn), copper (Cu), and cadmium (Cd) caused reduced arthropod density and microbial activity, resulting in a lowered rate of decomposition and a disturbance of forest nutrient dynamics.

An understanding of the mechanisms of toxicity is of practical importance. Knowledge on the mode of action provides a coherent basis for (a) interpretation of toxicity data, (b) estimation of the probability and the extent of harmful effects, (c) establishment of protocols to prevent toxic effects, and (d) discovering drugs that can counteract these effects. This chapter reviews the cellular and molecular mechanisms that contribute to the expression of toxicities. Complete mechanism of action information is rarely available and is not required for toxicity assessment. Mode of action is defined as the sequence of key cellular and biochemical events that result in a toxic effect, while mechanism of action implies a more detailed

understanding of the molecular basis of the toxic effect. Hence in this chapter it is important to distinguish between actions of metals and their effects. Actions of metals are the biochemical and/or physiological mechanisms by which the chemical produces a response in living organisms. The effect is the observable consequence of a toxic action. Although such mechanisms may be dealt with elsewhere in this book, an attempt is made to discuss this process in detail in this chapter in an integrated and complete manner with more emphasis on the mode rather than on the effect. This chapter further focuses integration of these mechanisms that have been identified definitively in animals and plants.

2 MODE OF ACTION AND TOXICITY OF TRACE ELEMENTS IN GENERAL

Hypothetically, the strength of a toxic effect of all trace metals depends principally on the absorption, concentration, and persistence of the eventual toxicant at its location of action. The final toxicant is the metal species that reacts with the endogenous target molecule such as receptors, enzymes, DNA, protein, or lipid or critically alters the biological environment, producing structural and functional changes that result in toxic damage. More often than not, the principal toxicant is the metal species to which the organism is exposed. However, in some cases the toxicant may be a metabolite of the parent compound or reactive oxygen or reactive nitrogen species (ROS or RNS) generated during the in vivo transformations of the toxicant. In few cases an endogenous molecule or compound synthesized as a response to primary toxicant exposure may be the principal toxicant. The buildup of the definitive toxicant at its target is facilitated by various biological processes that involve absorption, distribution to the site of action, reabsorption, and metabolic activation [Langman and Kapur, 2006]. Contrastingly, the predominant processes involved in counteracting toxic exposure contributing to tolerance are presystemic elimination, distribution away from the site of action, excretion, and detoxication which work against the accumulation of the ultimate toxicant at the target molecule. The logical steps by which toxicity results are typically as follows: delivery of toxicants > reaction of toxicants with target molecules > manifestation of dysfunction > counter reaction (repair)/failure of counter reaction (disrepair) > toxicity. Mode of action typically starts with the reaction of metals with target molecules and ends with toxic manifestations.

After mediation of toxicity by chemical reactions with a target molecule, a succession of secondary, tertiary, and quaternary biochemical events take place; this leads to dysfunction or injury that is apparent at diverse levels of biological organization, which may include the target molecule itself, cell organelles, cells, tissues and organs, the organism, and finally the community as a whole. Hence, mechanism of action and toxicity is typically characterized by the target molecules attributes, the reactions type's trace elements and target molecules, and the effects of trace elements on the target molecules. In addition, manifestation of toxicity may also be due to the alteration of the biological environment in which molecules,

cell organelles, cells, and organs operate in the absence of contact chemical reactions initiated by the toxicant.

The properties that make a molecule or substance qualify as a toxicant are (1) it reacts with the target and adversely affects its function, (2) it reaches an effective concentration at the target site, and (3) it alters the target in a way that is mechanistically related to the observed toxicity. Progression toward toxicity involves (1) the attributes of target molecules, (2) the types of reactions between ultimate toxicants and target molecules, and (3) the effects of toxicants on the target molecules [Gregus and Klaassen, 2001]. The reaction types after contact of the trace element and the target molecule would involve (i) noncovalent binding by the formation of hydrogen and ionic bonds involving the interaction of some trace metals with targets such as membrane receptors, intracellular receptors, and ion channels and (ii) covalent bonding by most traces metals that react with nucleophilic atoms that are plentiful in biological macromolecules, such as amino acids, proteins, and nucleic acids. Soft electrophilic metals such as silver and mercury react with (a) soft nucleophiles like sulfur in thiols, cysteinyl residues in proteins, and glutathione and sulfur in methionine (thiols and thiolates) and (b) hard electrophiles such as lithium, calcium, and barium, which react preferentially with hard nucleophiles (oxygen of purines and pyrimidines in nucleic acids phosphate oxygen in nucleic acids). On the other hand, chromium, zinc, and lead fall between these two extremes and exhibit universal reactivity with all nucleophiles including nitrogen in primary and secondary amino groups of proteins, nitrogen in amino groups, and in purine bases in nucleic acids. The reactivity of an electrophile determines which endogenous nucleophiles can react with it and become a target.

A typical target molecule having the functions of regulation, maintenance, and signaling when effectively attacked will initiate a cascade of effects as diversified as (a) changes in gene expression causing improper cell division apoptosis, (b) impaired protein synthesis, (c) changes in internal and external maintenance causing impaired ATP synthesis, and (d) altered membrane function leading to cell injury in plants and animals. Furthermore, in animals these changes may cause unbalanced homeostasis, bleeding, inappropriate neuromuscular activity-like tremors, convulsion, and paralysis. After the trace element has acted on the target, the last process would be the process of repair which, if absent, may cause damage at higher levels of the biological hierarchy in the organism, which in effect would be irreversible. The mechanisms of repair take place at molecular, cellular, and tissue levels. It is noteworthy that even repair mechanisms can contribute to the toxicity of trace elements; the best examples of this are (1) if excessive amounts of biomolecules are cleaved by the enzymes that assist in repairing broken DNA strands and (2) when too much reducing power is consumed for the repair of oxidized proteins and endogenous reductants.

Perturbations in signaling is one of the principal modes of action resulting in toxicity which is common to most trace elements (see Fig. 1). Oxidative processes enhancement resulting in the increase of superoxide anion radical ($O^{2\cdot-}$), H_2O_2, and hydrogen peroxide radical ($OH^{\bullet}$) is the base for other connections with signaling response. Oxidative stress affects numerous cellular components, such as DNA, lipids, and proteins, through oxidation reactions. These alterations in structure

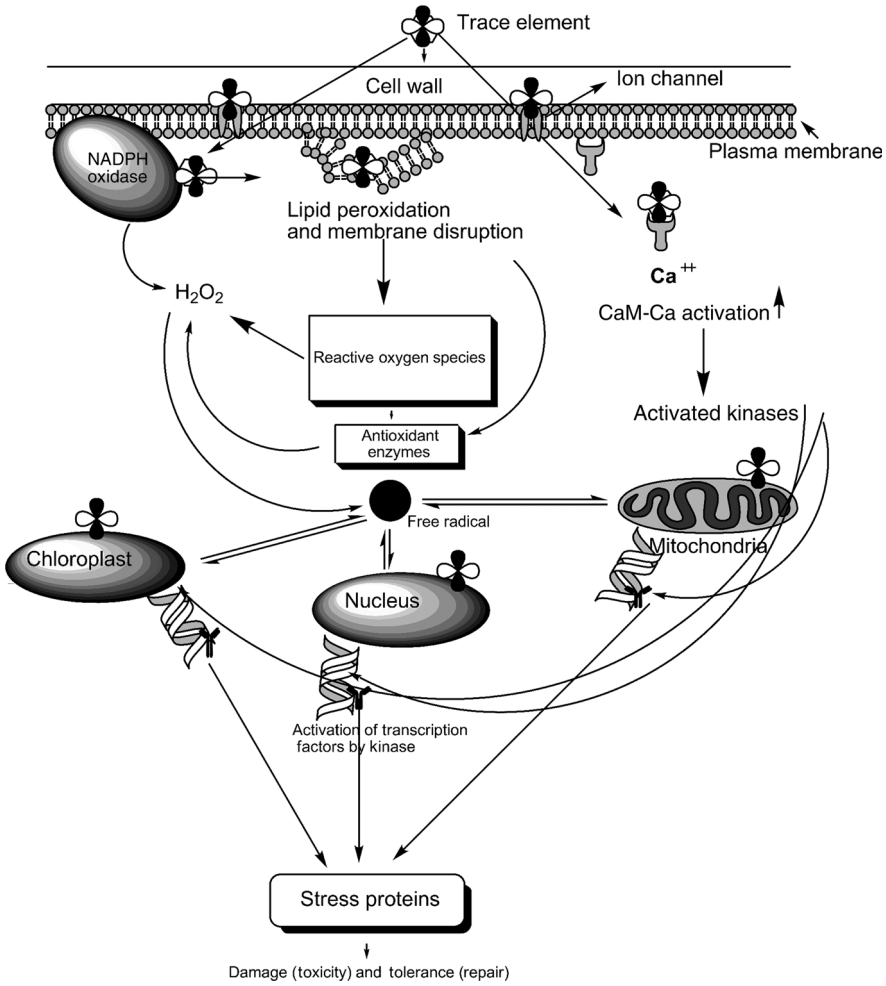

Figure 1. Generalized scheme of signaling induced by trace elements in living systems. In a very simplistic model of signaling systems leading toward toxicity or tolerance, the following would be the train of events. Trace element's entry in the system is facilitated by transport protein or diffusion. At the topical level there is increased production of H_2O_2 by direct action on NADPH oxidase; at the systemic level there is disruption of phospholipid bilayer due to lipid peroxidation, leading to production of ROS inducing synergistic action of SOD, CAT, and APx and increasing H_2O_2 levels especially by SOD. In due time course, excess metals enter cellular organelles like mitochondria and chloroplasts (plants), act as a sink in the electron flow, or misdirect the electron flow (depending on the redox status of the metal), which causes production of free radicals. Free radicals, in turn, initiate the antioxidant systems (Halliwel Asada Pathway) to quench H_2O_2. Unquenched H_2O_2 in addition to other free radicals gives rise to singlet oxygen. In addition, receptor metal complex in the plasma membrane causes excess calcium ion concentration which initiates the calmodulin-Ca^{2+} system activating various kinases. These reactive molecules and kinases act as signals on the transcription factors present in the nuclear as well as the organelle DNA, leading to the production of stress proteins and secondary metabolites that can act as either damage-causing agents or stress-countering agents.

produce significant changes in the function and may result in pathogenesis. Most trace elements produce reactive oxygen species, resulting in an increased lipid peroxidation (LPO), depletion of sulfhydryls, altered calcium homeostasis, and finally DNA damage. Oxidative stress refers to the cytopathological consequences of a disproportion between the production of free radicals and the capability of the cell to protect against them. Oxidative lipid injuries, named lipid peroxidation (LPO), create a progressive loss of membrane fluidity, thus reducing membrane potential and increasing its permeability to ions such as Ca^{2+}. Active oxygen species are continuously produced in tissues by (a) the action of the mitochondrial electron transport system and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and (b) photophosphyration in chloroplasts of plants, among other sources. There are several antioxidant defense systems, including the action of some enzymes, to antagonize oxidative damage; these include catalase (CAT) and superoxide dismutase (SOD) for hydrogen peroxide. This system is the primary line of defense by living organisms against trace elements.

A growing body of verification demonstrates that most trace metals mediate gene expression by activation of signal transduction pathways. Signal transduction is a progression by which information from an extracellular indication is transmitted into the cell through the plasma membrane and along an intracellular sequence of signaling molecules to excite a cellular response. The response may be the commencement of a gene transcription that occurs through various regulatory proteins that bind to specific DNA sequences in a gene. The net result of this binding is usually transcription of that gene and is referred to as transcriptional activation. Cell transformation is a complex process involving a variety of transcription factors and signaling pathways. Transcription factors, such as AP-1, NFκB, and the classical mitogen-activated protein kinase (MAPK) cascade, play important roles in cell proliferation, differentiation, and transformation [Jonak et al., 2004]. Apoptosis or cell death induced by most trace elements has been best studied. Apoptotic cascade pathways induced by metals have mitochondrial dysfunction as its originator. Mitochondrial dysfunction initiated by the opening of the mitochondrial transition pore leads to mitochondrial depolarization, release of cytochrome C, activation of a variety of caspases, and cleavage of downstream death proteins and ultimately results in apoptotic cell death.

3 SPECIFIC MODE OF ACTION OF MAJOR TRACE ELEMENTS

3.1 Arsenic

Arsenic is one of the few elements that is yet to be characterized as a single element. This is mainly due to its highly complex chemistry and also due to the existence of a plethora of compounds. It is trivalent or pentavalent and has a wide distribution. The most common inorganic trivalent arsenic compounds are arsenic trioxide, sodium arsenite, and arsenic trichloride. Pentavalent inorganic compounds are arsenic pentoxide, arsenic acid, and arsenates, such as lead arsenate and calcium arsenate.

Organic compounds may also be trivalent or pentavalent, such as arsanilic acid, or may even occur in methylated forms as a consequence of biomethylation by organisms in soil, fresh water, and seawater. Inhalation exposure to arsenic occurs in industrial settings such as lead, copper, and zinc smelting, from fossil fuel combustion in power plants, in the semiconductor industry, as well as in pesticide production. Nonoccupational exposure to arsenic can take place through ingestion of contaminated food and water. Contamination of well water with arsenic leached from underground sediments has occurred in large areas of India and Bangladesh, and hundreds of thousands of people have developed precancerous skin lesions due to arsenic ingestion [National Research Council Report, 1999]. High concentrations of arsenic in drinking water are also found within the United States in the western and southwestern states and in Alaska. Exposure to high concentrations of arsenic is associated with skin, lung, liver, and bladder cancers [Morales et al., 2000].

3.1.1 Mode of Toxic Action. The toxicity of arsenic is highly dependent on its oxidation state and chemical composition. Arsenite is taken into cells by passive diffusion, while arsenate competes with phosphate for uptake. Arsenite is extremely thiol-reactive. It can affect enzyme activities by binding to critical vicinal cysteinyl residues, such as those in the lipoamide of pyruvate dehydrogenase, tyrosine phosphatases, and enzymes involved in protein ubiquination. It is thought that arsenite is a sulfhydryl reagent having a high affinity mainly for vicinyl dithiols and also thiols located near hydroxyls. In contrast, arsenate is similar to phosphate in structure and may interfere with oxidative phosphorylation by forming an unstable arsenate ester. Thus, arsenate affects phosphotransfer reactions, which are required for ATP generation. Furthermore, arsenate is excreted more rapidly than arsenite from the body. Arsenite is therefore considerably more toxic and carcinogenic than arsenate. It is also believed that inorganic arsenic was more toxic than organic arsenic, and the methylation of inorganic arsenic was thought to be a detoxification process [Huang et al., 2004].

It is well established that the trivalent compounds are the principal toxic form of As and that pentavalent arsenic compounds have little or no effect. Sulfhydryl proteins and enzymes are extensively altered by exposure to arsenic. Reversal of alterations is possible in the presence of glutathoine in reduced or in oxidized form, and hence the Halliwell Asada pathway forms an important site for action and counteraction in As toxicity. The cellular toxicity of As is primarily because of impairment of mitochondrial enzymes and resultant blockage of oxidiative phosphorylation. The accumulation of As in mitochondria resulting in uncoupling of NAD linked substrates in the electron transport chain. Reactions between dihyrolipoic acid and arsenite ion prevents the oxidation of substrate. In addition, inhibition of succinic dehydrogenase by arsenite also contributes to increase in the activity of mitochondrial ATPase as a result of uncoupled oxidative phosphorylation. Arsenic inhibits energy-linked functions of mitochondria in two ways: (1) competition with phosphate during oxidative phosphorylation and (2) inhibition of energy-linked reduction of NAD. Inhibition of mitochondrial respiration results in decreased cellular production of ATP and increased production of hydrogen peroxide, which might cause oxidative stress,

and production of reactive oxygen species (ROS) (see Fig. 2 for a generalized scheme). Intracellular production of ROS results in observed induction of major stress protein families [Shi et al., 2004]. Arsenic compounds induce metallothionein in vivo. Potency is dependent on the chemical form of arsenic. As(III) is most potent, followed by As(V), monomethylarsenate, and dimethylarsenate [Kreppel et al., 1993].

Metallothionein is thought to have a protective effect against arsenic toxicity and may be responsible, at least in part, for its self-induced tolerance. Metallothionein-null mice are more sensitive than wild-type mice to the hepatotoxic and nephrotoxic effects of chronic or injected inorganic arsenicals [Liu et al., 2001]. In addition, in plants As clearly increases homophytochelatin (hPC) and phytochelatins (PC). It has been demonstrated that the formation of As–PC complexes is in accordance with a detoxifying role for the peptides [Gupta et al., 2004]. The role of asenical-induced oxidative stress and ROS may play a role in mediating DNA damage and initiating the carcinogenic process [Kligerman et al., 2005]. Although a well-recognized human carcinogen, arsenic itself is not a potent mutagen and has been thought to act through epigenetic mechanisms that modify DNA methylation

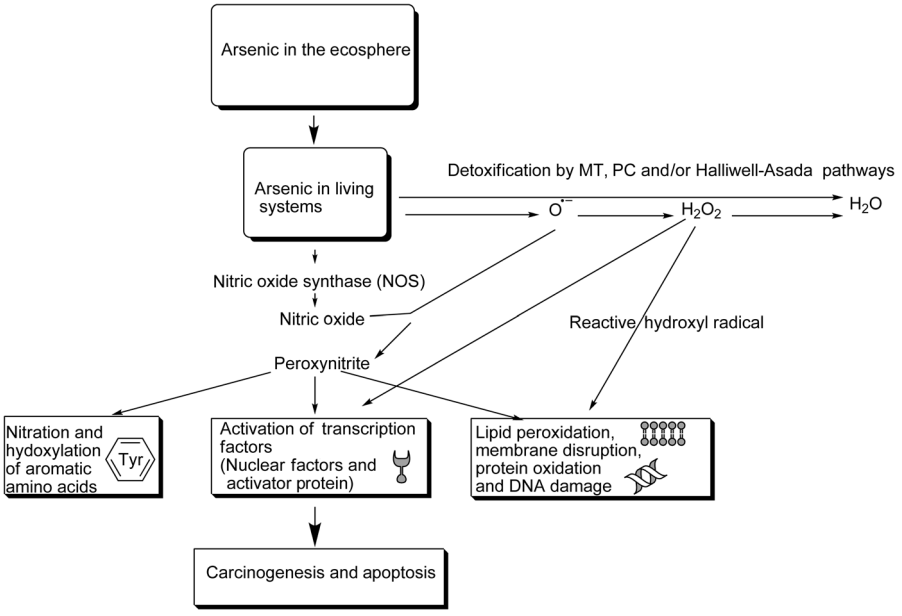

Figure 2. Schematic representation of arsenic mode of action in biological systems. Arsenic enters in vivo from the ecosphere and is either subjected to detoxification or proceeds to cause toxicity that is dose-dependent. Detoxification is mainly by antioxidant enzymes and metal-binding peptides which finally reduce the toxic free radicals to water. Unquenched and unscavenged ions generated by excess H_2O_2 and peroxynitrite cause nitration of aromatic amino acids (tyrosine) and activation of transcription factors that promote carcinogenesis and apoptosis.

patterns, perhaps in conjunction with DNA damaging agents. Hypomethylation of DNA is thought to be an early epigenetic mechanism that coincides with malignant transformation and a mechanism that transmits inappropriate gene expression patterns transgenerationally. Exposure to physiologically relevant concentrations of arsenic mediates hypomethylation of chromatin by competition for methyl donors and inhibition of DNA methyltransferase reactions that utilize *S*-adenosylmethionine as a cofactor [Reicharda et al., 2007].

The arsenite (+3) and arsenate (+5) forms have different modes of action. Arsenite binds to sulfhydryl groups and has been reported to inhibit over 100 different enzymes, while the arsenate can substitute for phosphate in various high-energy intermediates, resulting in arsenolysis. In addition, when arsenate is reduced to arsenite in vivo, it can also cause toxicity as that species. Recently, it has been demonstrated that chromosome fragments that are not incorporated into the nucleus at cell division (micronuclei formation) is the one of the principal manifestations of arsenic in plants and animals [Yi et al., 2007]. There are several possible hypotheses on the pathways of MN formation. The first pathway depends upon arsenic-induced oxidative stress. The induction of reactive oxygen species (ROS) was found in arsenic-exposed mammalian and plant cells [Requejo and Tena, 2005]. The attacks of ROS on purine and pyrimidine bases and on deoxyribose in DNA can cause DNA strand breakage, which can increase the probability of chromosome/chromatid fragmentation, thereby leading to the observed increases of MN formation. The second pathway involves the changes of protein sulfhydryl groups (−SH). Binding to sulfhydryl groups, arsenic can inactivate some important enzymes involved in DNA repair and expression, alter DNA repair mechanism, and cause an increase in MN frequency. There is some evidence to suggest that the cytoskeleton is an important cellular target of arsenic toxicity. Arsenic by interfering with microtubule assembly and spindle formation causes chromosomal lagging and leads to a higher frequency of micronuclated cells [Binet et al., 2006]. It has been reported that arsenite is a potent stimulator of proto-oncogene c-fos and c-jun expression and AP-1 transactivational activity. A DNA-binding protein composed of the Jun and Fos proteins, AP-1 regulates the transcription of various genes governing cellular processes, such as inflammation, proliferation, and apoptosis. Arsenic has been found to induce activation of JNKs, ERKs, and several other kinases. ERKs activation may contribute to the carcinogenic effects of arsenic, whereas JNKs activation is associated with apoptosis and results in the anti-carcinogenic effects of arsenic [Huang et al., 1999]. Although the mechanism by which JNKs mediates cell apoptosis in response to arsenite is not clear, several recent studies have shown that JNKs translocation to mitochondria is an important step in the resultant toxicity. JNKs play a central role in regulation of apoptosis and the release of cytochrome c and apoptotic proteases. Very recently, it has been found that JNK perturbation occurred during arsenite-induced malignant transformation, which is resistant to apoptosis as compared with passage-matched control cells, suggesting that apoptotic control mechanisms are disrupted as cells become transformed through arsenic exposure. This apoptotic disruption may allow damaged cells to inappropriately escape apoptosis and potentially proliferate, thereby providing initiating events in carcinogenic development. Thus,

this may lead to accumulation of genetically damaged cells that have a potential to become malignant [Huang et al., 2004]. Considering the fact that carcinogenesis is the principal toxic action of As, at present the mechanisms by which arsenic causes human cancers are not well understood. Arsenic is an atypical carcinogen because it is classified in neither the initiator nor the promoter categories of carcinogenic agents. Thus, arsenic probably does not act as a classical carcinogen, but rather enhances the carcinogenic action of other carcinogens.

3.2 Cadmium

Cadmium is a modern toxic metal. It was discovered as an element in 1817, and its industrial use was minor until about 50 years ago. But now it is a very important metal with many applications. Because of its noncorrosive properties, its main use is in electroplating or galvanizing. It is also used as a color pigment for paints and plastics and as a cathode material for nickel–cadmium batteries. Cadmium is a by-product of zinc and lead mining and smelting, which are important sources of environmental pollution. During the past century, Cd and its compounds have been used extensively in the smelting and electroplating industries and in the manufacturing of batteries, dyes, paints and plastics. Cadmium pollution was shown to have severe consequences on human, animal, and plant systems. Large amounts of Cd have also been released into the environment through the burning of refuse materials that contain Cd and through the use of Cd-contaminated sludge and phosphate salts as fertilizers. Tobacco contains significant amounts of Cd, and smoking is one of the primary sources of Cd exposure in the general population [Satarug and Moore, 2004]. Exposure to Cd can result in a variety of adverse effects in humans and animals. Depending on the dose, route, and duration of exposure, Cd can damage various organs including the lung, liver, kidney, bone, testis, and placenta. The most important effects are renal injuries, immune deficiencies, apathies, bone injuries, femoral pain, lumbago, and skeleton deformations. Cadmium exposure has detrimental effects on the CNS, with symptoms including headache and vertigo, parkinsonian-like symptoms, slowing of visuomotor functioning, peripheral neuropathy, decreased equilibrium, and decreased ability to concentrate. In addition, Cd has been shown to have teratogenic and carcinogenic activities.

Exposure to Cd has been associated with a wide variety of cardiovascular pathologies including hemorrhagic injury, atherosclerosis, hypertension, and cardiomyopathy [Navas-Acien et al., 2005]. The exact mechanism(s) through which cadmium produces its neurotoxic effects is still unresolved. Even though Cd represents a major environmental health problem, the specific mechanisms by which it produces its adverse effects have yet to be fully elucidated. Studies to address this issue have shown that Cd has a variety of biochemical, metabolic, and cytotoxic mechanism of action. However, in most cases, the relationships between these effects and the specific toxic actions of Cd in various target organs have not been firmly established. In plants, cadmium is a nonessential element, and the most evident symptoms of its toxicity are chlorosis and stunting. Chlorosis seems to be the result of the effects of Cd on the uptake, transport, and use of several elements (Ca, Mg, Fe, Mn, Cu, Zn, P,

and K), with the consequent reduction of Mn and Fe absorption and changes in Fe:Zn ratios. On the other hand, reduction of plant development seems to be the result of Cd interference with several important physiological processes: Cd alters the hormonal balance and disturbs the plant water status through a decrease of water absorption, reduction of root hydraulic conductivity into xylem vessels, decrease of transpiration rate, and increase of stomatal resistance [Mishra et al., 2006; Aina et al., 2007].

3.2.1 Mode of Toxic Action. Cadmium has various effects on molecules, cells, and organelles. Oxidative stress has often been discussed as a primary effect of Cd^{2+} exposure even though Cd is not a redox-active metal and will not take part in Fenton and Haber–Weiss reactions. Rather, symptoms of oxidative stress such as lipid peroxidation are a consequence of GSH depletion due to binding of Cd^{2+} to GSH and formation of GSH-derived PCs [Schützendübel and Polle, 2002]. Cell cycle progression, DNA replication and repair differentiation, proliferation, and apoptotic pathways are altered and affected by cadmium (Fig. 3). By activating

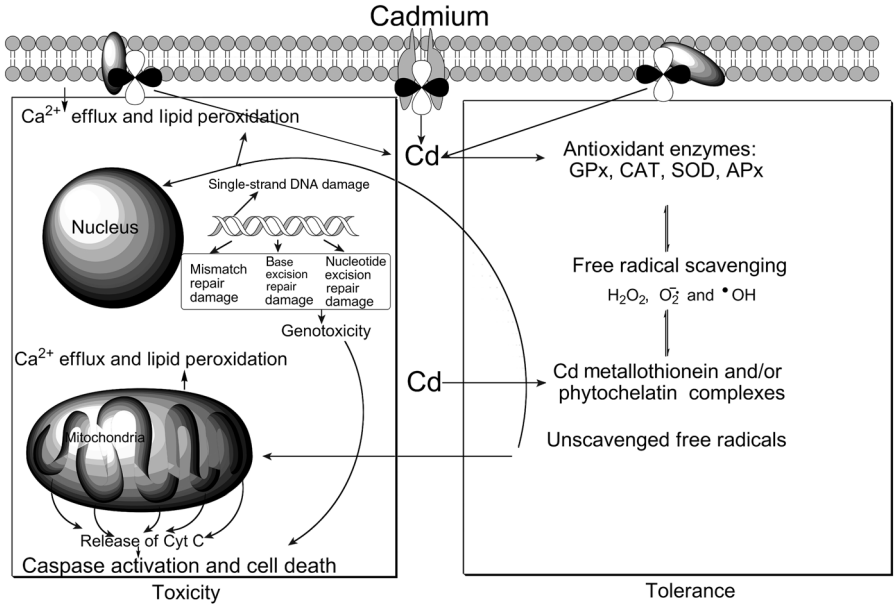

Figure 3. Scheme depicting cadmium-induced neurotoxicity and genotoxicity in cell. Cd ion enters cells mediated by channels or transport proteins. After entering, Cd induces a decrease or increase (in a dose-dependent manor) in the activities of scavenging enzymes. Metallothionein (MT) and/or phytochelatins are activated as scavengers of the free radicals. Excess free radical production increases the levels of lipid peroxidation and disruption of membranes [cellular, mitochondrial, and chloroplast (plants)]. Furthermore, Cd causes a decrease in intracellular ATP levels, leading to mitochondrial membrane breaking. This causes Ca^{2+} and cytochrome C to escape into the cellular matrix and activate various caspases by binding to Apaf-1 (apoptosis protease activating factor 1) and induce apoptosis and/or necrosis.

cellular signals, it regulates cell cycle progression and inhibition of DNA methylation and/or interference with cell adhesion. Almost all the effects including the effects on DNA synthesis and cell proliferation are clearly dose-dependent. Although it enhances DNA synthesis and cell proliferation at lower concentration than 1 μM, cadmium exposure above this concentration inhibits DNA synthesis and cell division [Yang et al., 2004; Dong et al., 2001]. RAS proteins are small enzymes, which serve as master regulators of a myriad of signaling cascades involved in highly diverse cellular processes. The RAS signaling pathway involved in cell proliferation and differentiation is one of the primary sites of cadmium action as a consequence of which many genes involved in cell cycle regulation are overexpressed and many proteins are up-regulated.

Cadmium also increases the level of several kinases of the RAS pathway, with MAPK being the one of the chief ones. Furthermore, cadmium treatment induces translocation of adhesion molecules and chaperones from the cytosolic side to the membrane side, thereby increasing DNA-binding activity of these molecules. Overexpression of the proto-oncogenes—namely, *c-fos*, *c-myc*, and *c-jun*—are induced by cadmium. These genes actively promote proliferation of cells and development of tumors after cadmium exposure. In mammalian cells, Cd^{2+} induces cyclin D and cyclin E expression by activation of a Myb-type transcription factor; in plants, Cd^{2+} causes inhibition of cyclin B [Deckert, 2005]. Several stress-inducible proteins such as hemeoxygenase-1(HO-1), regulating interleukin-10 (IL-10), and (tumor necrosis factor alpha (TNF-α) production are induced by cadmium. Cadmium also affects several genes involved in the stress response to pollutants or toxic agents.

Zn-dependent proteins and Zn-binding molecules are "candidate" targets of Cd^{2+} toxicity. The chemical similarity of these two ions makes it a possibility that Cd^{2+} ions can replace Zn and, in so doing, interfere with some of the many Zn reliant processes. Indirect confirmation for such consequences is the transcriptional up-regulation of recognized Zn^{2+} uptake systems, an observation that has been made in several biological systems [Weber et al., 2006]. It implies that a Zn^{2+}-sensing molecule can be engaged by Cd^{2+} ions as a result of Cd^{2+} exposure. Similarly, Ca^{2+}-binding proteins such as calmodulin might well be prime intracellular binding sites of Cd^{2+}, and such binding will most likely be detrimental to cellular signaling cascades. Still, in spite of a large body of work on metal toxicity, primary target sites of toxic metal effects in general, or Cd^{2+} in particular, remain to be identified [Clemens, 2006].

Metallothioneins are the most-studied proteins in relation to Cd and its mechanism of toxicity because it is readily induced in high quantities after cadmium exposure. Mammalian metallothioneins (MT) are cysteine-rich heavy-metal-binding proteins that can protect against cadmium toxicity and oxidative stress. They contain about 30% of cysteine residues, which are known for their ability to chelate free-cadmium. Intoxication of cells or animals with cadmium results in an increase of the production of metallothioneins, which belong to the principal pathway of detoxification of this heavy metal. The importance of metallothioneins is shown by the fact that administration of exogenous metallothionein to rats exposed to cadmium results in a decrease of oxidative stress. Metallothionein (MT) gene transcription is induced

by cadmium and oxidative stress. Metal response elements (MRE) present in the proximal promoters of MT genes and metal-responsive six zinc finger transcription factor MTF-1 are instrumental in the transactivation of MTs. The next important mode of action relates to the effects of Cd on heat shock proteins (HSPs). Cadmium intoxication alters the expression levels of HSPs, which are active stress-responsive proteins. After Cd^{2+} exposure, overexpression of the genes encoding HSPs has been widely reported. HSPs are cellular chaperone proteins, which can be induced by various environmental stresses including toxic exposure to cadmium. HSP induction is generally considered as an adaptive response of cells to stress, closely linked with cell survival. HSP proteins participate not only in a principal role of chaperoning folding but also in the degradation of proteins. In addition, apoptosis pathways are also activated or inhibited by HSPs. Cd^{2+}-mediated induction of HSPs is largely due to its effects on proteins. Denaturation and oxidation of proteins by cadmium are responsible for the overexpression of HSP chaperones [Gaubin et al., 2000]. HSPs induction after Cd^{2+} exposure is also linked to the increase of oxidative stress. Cadmium intoxication induces overexpression of at least 10–15 families of heat shock chaperone proteins.

Apoptosis is one of the well-studied effects of Cd in living tissues other than plants. Cell death resulting from cadmium (Cd) intoxication has been confirmed to occur through apoptosis by morphological and biochemical studies. However, it is still not clear whether Cd itself or metallothionein (MT) induced by Cd is the major factor responsible for the apoptosis. CdMT may play a paradoxical role, providing protection against the cadmium ion in the intracellular milieu, but promoting cadmium toxicity when it is present in sufficient amounts in the parts of living systems where absorption is fast. Thus, the function of MT in relation to the effect of Cd is rather debatable. As discussed above, oxidative damage due to Cd is a well-proven mechanism of action. Taking these together, it can be said that Cd causes apoptosis through its indirect oxidative effects routed through overexpression of MT genes. Three apoptotic pathways have been described: (1) mitochondria-dependent pathway, (2) death-receptor-dependent pathway, and (3) endoplasmic reticulum (ER) pathway. There is an individual initiator caspase in each apoptosis pathway: caspase-9, in the mitochondrial pathway; caspase-8, in the death-receptor-dependent pathway; and caspase-12, in the ER pathway. Cadmium probably induces apoptosis through the mitochondrial pathway; Lopez et al. [2006] showed that the decreases in the ATP intracellular levels at the highest concentration of cadmium were accompanied by ATP release, indicating mitochondrial and cytosolic membrane breaking. This mechanism, produced by mitochondrial toxicity with fall in ATP, breakdown of mitochondrial membrane potential, and ROS formation, induces apoptosis with disruption of cellular membranes and necrosis.

Nerurotoxicity is also induced by Cd, and it is known that oxidative stress caused by Cd in nerve cells completely blocks CNTF family neurokinase and IFN-γ-mediated Jak/STAT signaling; this seems to be a novel mechanism for mediating cadmium neurotoxicity [Monroe and Halvorsen, 2006]. It seems that the administration of cadmium initially affects the integrity and permeability of the vascular endothelium, and necrotic changes in nerve cells occur secondarily to this effect.

Cadmium inhibits all of the known pathways of cellular Ca^{2+} influx and acts as a competitive ion to Ca^{2+} at the voltage-dependent Ca^{2+} channels, and it is a potent blocker of the Ca^{2+}-dependent neurotransmitter release. This effect on Ca^{2+} influx is due to the interaction of the heavy-metal ion with thiol groups of proteins involved in intracellular Ca^{2+} sequestration. On the other hand, Cd has been reported to elevate the intracellular Ca^{2+} concentrations. This sustained increase is believed to be the main cause for cellular death. In addition, an increase of free radicals and Ca^{2+} levels associated with Cd exposure induces mitochondrial disruption and release cytochrome C into the cytosol. The change of the membrane potential of the mitochondria membrane opens the transition pore, stimulating again the release of cytochrome c. Cytochrome c is a component of the electron transport chain and is involved in the production of ATP; cytochrome c activates caspases by binding to specific proteins, inducing it to associate with procaspase-9 (holoenzyme), thereby triggering caspase-9 activation and initiating the proteolytic cascade and eventually cell death. In addition to causing cell death, neurotoxicty, and genotoxicity, cadmium targets the DNA repair mechanism itself, thus posing as a great threat among all the known toxic trace elements.

DNA repair is major protection machinery against DNA injury caused by regular metabolic activities and environmental factors. It includes a multiplicity of biochemical mechanisms that add to the preservation of genetic sequence, minimizing cell killing, mutations, duplication errors, persistence of DNA harm, and genomic volatility. Cadmium interferes with multiple DNA repair process, and at low biological relevant concentrations it enhances the mutagenicity induced by other DNA-damaging agents. Cadmium (a) interferes with base excision repair (BER) by reducing the repair capacity of formamidopyrimidine DNA glycosylase, (b) interferes with nucleotide excision repair (NER) by disturbing in a dose-dependent manner, DNA-protein interactions essential for the initiation of NER, and (c) interferes with mismatch repair (MMR) by inhibiting the capacity of the MMR process to correct base–base and insertion/deletion mismatches, which had escaped the proofreading function of replicative polymerases. It can thus be postulated that this interferences with DNA repair mechanisms is the cause of many other effects of Cd on living systems. As Giaginis et al. [2006] have suggested, an ample amount of evidence shows that Cd might interfere with DNA repair procedure, leading to increased buildup of damaged DNA bases. The failure of DNA repair systems to correct affected bases can lead to harmful mutations, genomic instability, or cell death. In higher eukaryotes, the damage that occurs in DNA stability genes accountable for DNA repair and cell cycle control can result in tumor creation. Although Cd is mainly implicated in the initial steps of DNA repair process, additional indications suggest that further steps could also be inhibited. Specifically, it has been established that many proteins, involved in these DNA repair systems, are possible targets of Cd toxicity. Furthermore, it is well known that Cd is able to complex with DNA repair proteins either by substitution of Zn from their Zn finger motif or by binding to negatively charged surface residues on their structure, leading to conformational changes and disturbing the DNA–protein interactions, essential for DNA repair process and maintenance of the genome integrity. The complete, detailed, step-by-step

mechanism by which Cd inhibits DNA repair is yet to be elucidated, and this essentially contributes to the lack of explicit understanding of Cd-induced mutagenicity and carcinogenicity.

3.3 Chromium

Chromium (Cr) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquelin. It is a transition element located in group VIB of the periodic table with a ground-state electronic configuration of $Ar3d^5 4s^1$. The stable forms of Cr are the trivalent Cr(III) and the hexavalent Cr(VI) species, although there are various other valence states that are unstable and short-lived in biological systems. Cr(VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions. Cr(III) is less mobile and less toxic and is mainly found bound to organic matter in soil and aquatic environments. Cr(III) is a micronutrient important in the biological activity (receptor binding) of insulin and, accordingly, can be found in many dietary supplements. Contamination of soil and groundwater due to the use of Cr in various anthropomorphic activities has become a serious source of concern to plant and animal health over the past decade. It is estimated that several hundred thousand workers are potentially exposed to high levels of Cr(VI). Occupational exposure to Cr (Cr(III) and Cr(VI)) by inhalation depends upon the job function and industry, but can reach several hundred micrograms per cubic meter. These estimated exposures have been significantly lowered in the past few decades as industrial hygiene practices and worker controls have been implemented. Nonoccupational exposure to Cr occurs from automobile emissions and cigarette smoke.

It has been estimated by IARC that, on average, cigarettes produced in the United States contain 0.24–6.3 mg Cr/kg. In the environment, elevated levels of Cr have been reported in areas near landfills, hazardous waste disposal sites, chromate industries, and highways. Chromium, in contrast to other toxic trace metals like cadmium, lead, mercury, and aluminum, has received lesser attention from scientists. Its complex electronic chemistry has been a major hurdle in unraveling its toxicity mechanism in general. The impact of Cr contamination in biological systems depends on the metal speciation, which is responsible for its mobilization, subsequent uptake, and resultant toxicity in the biological systems. Chromium toxicity in plants is observed at multiple levels in plants and animals, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis [Shanker et al., 2005; O'Brien et al., 2003].

3.3.1 Mode of Toxic Action. Chromium(VI) has long been recognized as a carcinogen in human and mammalian systems. Since hexavalent chromium does not damage DNA in vitro without a reducing agent, it is believed that the actual mutagenic species is one or more of the reactive intermediates produced in the reduction of Cr(VI) to Cr(III) (see Fig. 4). Of the many reducing agents available in the cellular environment, glutathione (GSH) is suspected to be a prime reductant due to its fairly high concentration in the cytosol, its favorable reduction potential,

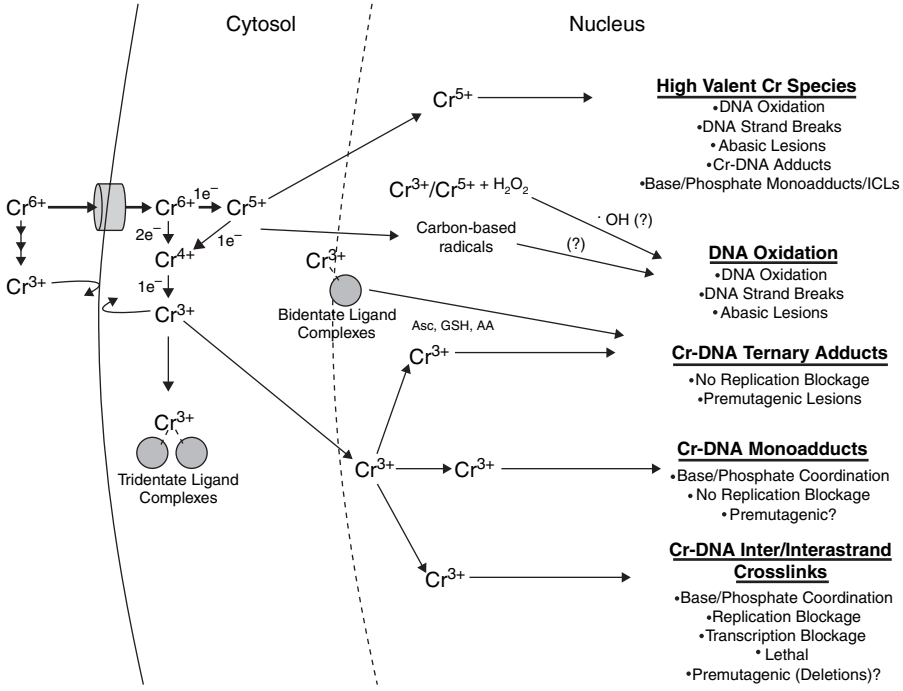

Figure 4. Major pathways involved in the formation of genetic lesions by Cr. This scheme illustrates the interrelationships between Cr metabolism and genotoxicity. Cr^{6+} enters the cell via anionic transporters and is rapidly reduced by a one- or two-electron mechanism to Cr^{3+} which cannot cross the cell membrane. An initial one-electron reduction can lead to the generation of high valent Cr species (Cr^{5+}/Cr^{4+}). Both Cr(III) and Cr(V) display an appreciable affinity for both DNA bases and the phosphate backbone leading to the formation of Cr–DNA monoadducts. Cr^{5+} can directly oxidize DNA bases and sugars and produce DNA strand breaks. Cr^{3+} is the ultimate DNA reactive species and is critical for the formation of DNA adducts. Both Cr^{3+} and Cr^{5+} may generate the hydroxyl radical in the presence of elevated levels of H_2O_2 and lead to "oxidative" DNA damage. Note that the relative location of some reactions/species is not intended to imply that these occur exclusively within that intracellular compartment. (Adapted from O'Brien et al. [2003] with kind permission from Elsevier.)

and its ability to produce long-lived Cr(V/IV) intermediates during the reduction of Cr(VI). Glutathione Cr interactions in plants have been fairly well elucidated. Chromium stress can induce three possible types of metabolic modification in plants: (i) alteration in the production of pigments that are involved in the life sustenance of plants (e.g., chlorophyll, anthocyanin), (ii) increased production of metabolites [malondialdehyde (MDA), H_2O_2 (see Fig. 5) glutathione, ascorbic acid] as a direct response to Cr stress which may cause damage to the plants [Shanker, 2003], and (iii) alterations in the metabolic pool to channelize the production of new biochemically related metabolites which may confer resistance or tolerance to Cr stress (e.g., phytochelatins, histidine).

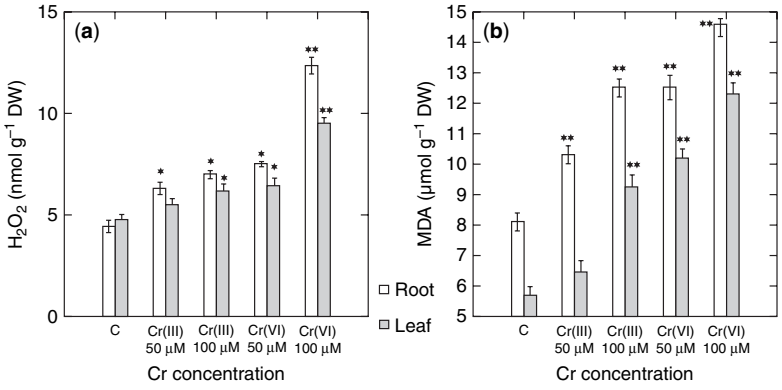

Figure 5. Levels of H_2O_2 (**a**) and lipid peroxidation expressed as malondialdehyde (MDA) (**b**) in roots and leaves of sorghum treated with different concentrations of Cr(III) and Cr(VI). Data represent mean $\pm$ SE of five separate experiments. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

Glutathione pool dynamics of plants, in terms of GSH and GSSG and the GSH/GSSG ratio, is affected by Cr speciation stress (Fig. 6), indicating the role of this pathway in the mechanism of action of Cr [Shanker and Pathmanabhan, 2004]. There is a marked decline in the GSH pool under Cr speciation stress more severely in roots (Fig. 5). Chromium-induced oxidation has been observed in different cellular thiols such as GSH and cysteine by Cr(VI) in in vitro studies. Dichromate reacts with GSH at the sulfhydryl group forming an unstable glutathione–CrO_3^- complex. Thiolate complexes of Cr(VI) with g-glutamylcysteine, *N*-acetylcysteine, and cysteine have also been described. The interconversion of reduced and oxidized forms of glutathione to maintain redox status of the cell as well as to scavenge free

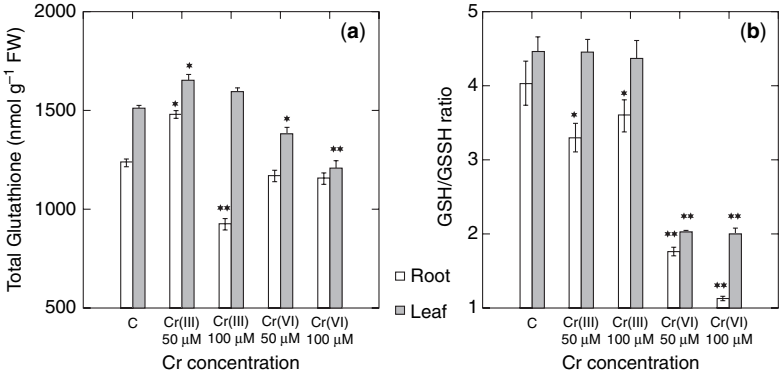

Figure 6. Levels of total glutathione (**a**) and GSH/GSSG ratio (**b**) in roots and leaves of sorghum treated with different concentrations of Cr(III) and Cr(VI). Data represent mean $\pm$ SE of three separate experiments. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

radicals could be one of the roles of GSH under Cr stress. Metal-binding peptides like metallothionein have been reported to have increased under Cr(VI) stress [Shanker et al., 2004b]. Q1

The role ROS scavenging enzymes have been clearly elucidated by Shanker et al. [2004a] in plant parts under both Cr(III) and Cr(VI) stress in plants. Scavenging enzymes are unaffected by lower concentration of Cr(III) because there is insignificant ROS production. The combined action of SOD and CAT is critical in mitigating the effects of oxidative stress, since the former merely acts on the superoxide anion converting it to another reactive intermediate (H_2O_2) and the latter acts on H_2O_2 by converting it to water and oxygen. SOD, in contrast to CAT, is more active in scavenging chromium-induced ROS. APX, which essentiality catalyzes the same reaction as CAT, compensates for the reduced CAT activity in plant roots. This could be because unlike CAT, which is present only in the peroxisome and has low substrate affinities since it requires simultaneous access of two molecules of H_2O_2, APX is present throughout the cell and has higher substrate affinity in the presence of ascorbic acid as a reductant. Cr(VI), apart from generating ROS which consequently sets off a signaling response for active scavenging, could actually inhibit the scavenging enzyme activities at acute concentrations. The high contents of dihydro ascorbate (DHA) in combination with an absence of active scavenging and blockage of normal cell cycle progression by DHA is one of the main mechanisms for inducing toxicity in plants by Cr. The role of GSH is more of a signal intermediate rather than direct participation in detoxification of ROS. This mechanism is more likely because of the reported absence of phytochelatin production under Cr stress and because glutathione serves as a precursor for phytochelatin production. The cellular redox status is maintained by the ratio of GSH/GSSG.

The GSH–GSSG redox pair can function effectively only when there is an adequate supply of NADPH and because GSH itself can serve as a cellular sensor to maintain the NADPH pool. Cr(VI) can function as a hill reagent and can inhibit electron transport both in the photosynthetic and mitochondrial apparatus, thus accounting for reduced NADPH pool. The critical balance between the available NADPH pool and ROS production by Cr would decide the redox status of the cell in both plants and animals. Chromium–DNA interactions are the one of the well-explained mechanisms of action of Cr in causing cell death and cancer. There are three theories that account for Cr-induced DNA injury, all of which involves ROS. First, Cr(VI), or reactive intermediate species (Cr(VI)/Cr(III)), may participate in a "Fenton-like" reaction with H_2O_2 leading to hydroxyl radical production. Second, reaction of Cr(VI) with hydrogen peroxide produces the superoxide anion and hydroxyl radical and leads to ROS-mediated DNA strand breaks. Third, oxidized DNA bases (and ROS) generated by the metabolic reduction of Cr(VI) has been suggested to be related to the production of metal-mediated mechanisms DNA injury.

Cr(VI)-containing compounds are genotoxic and can induce gene mutations, sister chromatid exchanges, and chromosomal aberrations. Cr(VI) alone does not react with isolated DNA, because the cellular constituents of reductive metabolism must be present for Cr to damage macromolecules. Chromium associates with both DNA bases and the phosphodiester backbone and the binding occurs through both

coordinate covalent binding or electrostatic/ionic interactions. The base-specific binding of Cr has revealed a general, but not absolute, preference toward the formation of Cr(III)–guanine DNA adducts (a DNA adduct is an abnormal piece of DNA covalently bonded to a carcinogenic chemical) and polyriboguanylic acid (poly(G)) in the case of RNA [O'Brien et al., 2003]. The major mechanism involved in the formation of genetic lesions by Cr is explained in Fig. 5. DNA protein cross-links (DPCs) are also one the modes by which Cr induces cytotoxic effects. Cr(VI), and not Cr(III), salts cross-link proteins to DNA in mammalian cells. Unlike nickel, which catalyzes DPC formation largely through oxidative mechanisms, Cr(VI) directly couple proteins to DNA. Cr–DPCs have been reported to extensively develop respectively between DNA and nonhistone proteins and RNA and cytoplasmic proteins in many animal systems [Reem et al., 2007].

Consistent with their ability to generate DNA strand breaks, Cr(VI)-containing compounds are well-documented clastogens. Clastogens are chemical agents that increase the rate of genetic mutation by interfering with the function of nucleic acids. Clastogens are usually specific mutagens that cause breaks in chromosomes. In most cases, damaged metaphases have been observed for both water-soluble and insoluble chromates. Evidence also indicates that chromosomal abnormalities (micronuclei) and genomic instability (microsatellite instability) are possibly involved in the induction of cancer by Cr(VI). DNA interstrand cross-links (ICLs) are caused by Cr(III) interacting with reaction centers on the complementary strand of DNA. DNA adducts are covalent adducts between chemical mutagens and DNA. Such couplings activate DNA repair processes and, unless repaired prior to DNA replication, may lead to nucleotide substitutions, deletions, and chromosome

TABLE 1. Apoptosis Induced by Ar, Cd, and Cr and Their Possible Mechanism of Action

Metal	Compound	Apoptosis target or Possible Mechanism
Arsenic	$NaAsO_2$	Apoptosis by activation of caspase-3, apoptosis by increase in MAPK, JNK, and p38 phosphorylation in T lymphocytes.
	As_2O_3	Apoptosis by disruption of mitochondrial membrane in acute promyelocytic leukemia cells.
Cadmium	$CdCl_2$	Apoptosis by breakdown of mitochondrial membrane and activation of caspase-9 and rapid phosphorylation of MAPKm-induced activation of JNK.
	$Cd(C_2H_3O_2)_2$	Apoptosis in rat primary epithelial lung cells by increase in protein expression in rat primary epithelial lung cells.
Chromium	Na_2CrO_4	Apoptosis by increased release of cytochrome c, depressed oxygen consumption, and decrease in mitochondrial NADH level.
	$K_2Cr_2O_7$	Apoptosis in human lung epithelial cells by reduced mitochondrial membrane potential and increase in p53 protein level and ROS in general.

rearrangements. Chromium complexation with glutathione and other metabolites of ROS induce the formation of DNA adducts, leading to genotoxicity. The relative role of reactive oxygen species (ROS) to the mutagenicity and carcinogenicity of Cr(VI) is not implicit and is still a topic of research by many groups. A notion that has received much attention is that intracellular Cr(VI) mediates Fenton-like reaction and produces ROS that are responsible for nearly all of the toxicity and genotoxicity caused by Cr(VI) [Shanker et al., 2005]. This has led some to assume that cells respond to Cr(VI)—and subsequent production of high valent Cr(V), ROS, and organic radicals—with a classic oxidative stress response. Table 1 gives an overview of the apoptosis induced by Ar, Cd, and Cr and their possible mechanism of action.

4 SPECIFIC MODE OF ACTION OF OTHER METALS

4.1 Nickel

Nickel was discovered by Cronstedt in 1751, and it is the 24th element in order of natural abundance in the earth's crust. It is widely distributed in the environment. Natural sources of atmospheric nickel include dusts from volcanic emissions and the weathering of rocks and soils. Natural sources of aqueous nickel are derived from biological cycles and solubilization of nickel compounds from soils. Global input of nickel into the human environment is approximately 150,000 metric tonnes per year from natural sources and 180,000 metric tonnes per year from anthropogenic sources, including emissions from fossil fuel consumption, and the industrial production, use, and disposal of nickel compounds and alloys [Kasprzak et al., 2003]. The consumption of nickel-containing products leads to environmental pollution by nickel and its by-products at various stages of production, recycling, and disposal. Human exposure to nickel occurs primarily via inhalation and ingestion. Wearing or handling of jewelry, coins, or utensils that are fabricated from nickel alloys or that have nickel-plated coatings may result in cutaneous nickel absorption. Occupational exposure to nickel occurs predominantly in mining, refining, alloy production, electroplating, and welding. In 1990, the International Committee on Nickel Carcinogenesis in Man suggested that respiratory cancer risks are primarily related to exposure to soluble nickel concentrations above $1\,mg/m^3$ and to exposure to less soluble forms at concentrations above $10\,mg/m^3$. Significant amounts of nickel in different forms may be deposited in the human body through occupational exposure and diet over a lifetime. Since nickel has not been recognized as an essential element in humans, it is not clear how nickel compounds are metabolized.

4.1.1 Mode of Toxic Action. Very similar to other metals, nickel too induces oxidative stress that depletes glutathione and activates Ap1, NF-kB, and other oxidatively sensitive transcription factors. The molecular basis of nickel carcinogenesis has proved vague since carcinogenic nickel compounds are inadequately mutagenic in most assay systems even though they produce oxidative DNA damage and inhibit DNA repair activity. Various compounds of nickel vary

in their activity to produce carcinogenic effects due to differences in their uptake, transport, distribution, retention, and the capacity to deliver Ni ions to specific cells and target molecules. Nickel compounds generate specific morphologic chromosomal damage. However, this may not be sufficient to produce mutations. Q2

Hypoxic signaling pathway activation by nickel has emerged as a new theory to explain toxic manifestation of nickel. Nickel substitutes for iron in a hypothetical oxygen sensor, thus switching a cell's metabolism to a state that mimics permanent hypoxia. Since hypoxia is common in solid tumors and selects for more malignant phenotype, this information provides new prospects for understanding the molecular mechanisms of nickel carcinogenesis. The exposure of cells to nickel triggers cellular reactions typical of hypoxia, including the expression of genes involved in glucose transport and intracellular metabolism. The GeneChip microarray technique revealed that genes coding for glycolytic enzymes, glucose transporters, and other hypoxia-inducible genes regulated by hypoxia-inducible factor 1 (HIF-1) are induced by nickel [Salnikow et al., 2003]. Additionally, cellular responses to hypoxic stress include inhibition of cell proliferation and, when cell damage is irreversible, apoptosis. Further progress in understanding molecular mechanisms of nickel carcinogenicity has been achieved in a study showing that nickel compounds increase the extent of DNA methylation that leads to the inactivation of gene expression.

Nickel interacts with DNA by replacement of Zn^{2+} with Ni^{2+} on the Zn^{2+}-binding sites of DNA-binding proteins. Ni^{2+} has an ionic radius similar to that of Zn^{2+}. DNA-binding proteins or "Zn finger loops" have been identified on some proto-oncogenes and are thought to be likely targets for metal toxicity. There are possibly two mechanisms by which cell transformations are caused by nickel although being weakly mutagenic. One method is by DNA damage and the other method is by epigenetic changes exerted by nickel compounds and the induction of cytosine methylation and histone deacetylation which leads to the inactivation of the senescence/tumor suppressor gene(s). Synergistic action of nickel with many mutagenic carcinogens in enhancing cell transformation both in vitro and in vivo is also known. Nickel-induced carcinogenesis is known to be both tissue- and species-dependent. All these theories of mechanism of action of nickel suggests that genetic predispositions, including variations in metabolism and antioxidant capacities of different species and strains of animals, may also play an important role in nickel carcinogenesis. The possible role of oxidative pathogenesis caused by nickel also has been explained. As compared with copper, iron, cobalt, and other redox-active metals, nickel produces relatively low, but measurable, levels of ROS in cells The oxidative effects of nickel depend on its ability to form the Ni(III)/Ni(II) redox couple at around pH 7.4 when Ni(II) is complexed by some natural ligands, including peptides and proteins. A number of proteins with high affinity to nickel have been identified in recent years. They are mainly involved in nickel transport, detoxification, and excretion.

The capacity of nickel ions to interact with a number of proteins raises the likelihood that nickel may appreciably change intracellular homeostasis by altering protein functions and producing stress comparable to unfolded protein response. Unlike most other metals, the metal-binding protein metallothionein does not

appears to constitute a major nickel-binding component in different tissues. Change of the oxidation status of redox-dependent regulatory proteins by nickel complexation may disturb the timely and orderly generation of cellular messengers like oncogenes, tumor suppressor genes, and many others, eventually resulting in improper progression of the cell cycle and/or apoptosis. In addition, DNA–protein cross-links, oxidative damage, and the inhibition of nucleotide excision repair which have been implicated in metals described above are also believed to be some of the mechanisms of nickel-induced cellular toxicity. Molecular mechanisms of nickel toxicity are still in its nascent stage as compared to the degree of progress with respect to other, more toxic metals.

4.2 Lead

Lead is an omnipresent toxic metal and is detectable in practically all phases of the inert environment and in all biological systems. Lead is one of the oldest-established poisons. Knowledge of its general toxic effects stretches back three millennia, and knowledge of its effects in children spans over 100 years. The major issue regarding lead is determining the dose at which it becomes toxic. Since it is toxic to both plants and animals at high exposures, there is no proven biological need for it. In human health, lead affects your children most. Exposure to lead at concentrations lower than recognized as threshold by the Center for Disease Control and Prevention adversely affects cognitive, neurobehavioral, and neurophysiological development of young children. The main route of contact for humans and animals is food, and sources that produce excess exposure and toxic effects are usually environmental and presumably controllable. These sources include lead-based indoor paint in old dwellings, lead in dust from environmental sources, lead in contaminated drinking water, lead in air from combustion of lead containing industrial emissions, hand-to-mouth activities of young children living in polluted environments, and lead-glazed artifacts.

4.2.1 Mode of Toxic Action. Possible mechanisms for lead toxicity include competition with and substitution for calcium, disruption of calcium homeostasis, stimulation of release of calcium from mitochondria, and opening of mitochondrial transition pore. In addition, direct damage to mitochondria and mitochondrial membranes by generation of ROS is also seen. Disruption of tissue oxidant/antioxidant balance, alteration of lipid metabolism, and substitution for zinc in various zinc-mediated processes are some of the metabolic repercussions of lead toxicity [Ahamed et al., 2007]. Lead toxicity development due to calcium interaction has gained considerable amount of attention by researchers. Calcium blocks the uptake of lead through the intestine because lead is a strong blocker of calcium channels.

Lead and calcium compete for the same binding sites on a large family of ion-binding proteins composed of calmodulin and related proteins. Calmodulin serves as a sensor for the concentration of calcium within cells. Lead acts by displacing calcium ions bound to calmodulin. Lead impairs normal calcium homeostasis and uptake by calcium membrane channels and substitutes for calcium in calcium

sodium ATP pumps. Impact of lead on brain activity is explained by the fact that lead also blocks access of calcium into nerve terminals, thereby blocking calcium movement into the mitochondria of brain cells, resulting in a decrease in energy production to perform brain functions. Lead also blocks heme synthesis, thereby increasing levels of the precursor daminolevulinic acid (ALA). ALA suppresses GABA-mediated neurotransmission by inhibiting its release and also possibly by competing with GABA at receptors. Lead also can produce anemia, both by interfering with heme synthesis and by decreasing iron absorption from the gut. Lead's ability to substitute for zinc, mentioned previously, affords another avenue by which lead can act as a neurodevelopmental toxicant. By displacing zinc, lead can alter the regulation of genetic transcription through sequence-specific DNA-binding zinc finger protein or zinc-binding sites in receptor channels [Lidsky and Schneider, 2003].

4.3 Mercury

Mercury is a metal that is a liquid at room temperature. The chemical symbol, Hg, is derived from "hydrargyros," the Greek word for "water silver." Mercury is generally found in three oxidation states, each with a specific toxic profile. In the zero oxidation state, Hg^0, elemental mercury exists as the liquid metallic form or as vapor. The two other inorganic forms of mercury are the mercurous (Hg^+) or the mercuric (Hg^{2+}). The mercuric state also forms organic compounds such as methyl, ethyl, phenyl, or dimethyl mercury. Mercury is used as a component of barometers, thermometers, dental products (amalgam), and electrical equipment, as well as in pest control chemicals and in fungicides. It is also used in the gold industry. Mercurous chloride is one of the oldest known pharmaceuticals and is continuously used for its antiseptic properties. It prevents fungus contamination in seeds, and it is good to amalgamate other metals. Cinnabar (mercuric sulfide), the natural form of mercury, was used for the red coloring in cave drawings thousands of years ago. Tombs adorned with elemental mercury have been noted in Egypt dating back over 3500 years.

Mercury's medicinal use probably originated in China and India nearly 2000 years ago. Medications containing this element have been used as antibacterial (syphilis), antiseptics, dermatologic ointments, teething compounds, laxatives, and diuretics. In the United States in the 1800s, the etiology of an epidemic within the hat industry referred to as "hatters' shakes" or "Danbury shakes" resulted from mercury exposure that occurred during production of felt. Exposure to Hg occurs via inhalation, ingestion, and parenteral or subcutaneous administration. Exposure to the vapor may come from sources including the burning of fossil fuels, emissions from volcanic activity, smelting processes in mining activities, the industrial electrolytic production of HCl and NaOH, industrial and medical waste incineration, degassing from the natural erosion of the earth's crust, evaporation from water, vaporization from dental amalgams, and crematoriums [US EPA, 1997]. Table 2 gives a summary of mode of action of Lead, Mercury and Nickel in brief.

4.3.1 Mode of Toxic Action. Inorganic mercurials and organomercury compounds have different modes of action due to the difference in their chemical structure and reactivity. The principal feature of organomercurials is the presence

TABLE 2. Summary of Mode of Action of Lead, Mercury, and Nickel

Metal	Position in Periodic Table	Atomic Number and Mass	Electronic Configuration and Valence State	Available Forms	Principal Use	Mode of Action on Biological Compounds
Lead (Pb)	IVA	82 and 207.19	$+2, +4$ [Xe] $4f^{14}5d^{10}6s^{2}6p^{2}$	Oxides, sulfides, acetates, chlorates, and chlorides	Used in solder, shielding against radiation and in batteries.	Lead binds strongly to a large number of molecules like amino acids, several enzymes, DNA, and RNA; thus it disrupts many metabolic pathways. Common ligands attacked are (γGlu–Cys)2Gly, (γGlu–Cys)3Gly, (γGlu–Cys), cysteine, acid-soluble thiol, and glutathione. Direct damage to mitochondria. Calcium channel blocks. Displace Zn in Zn finger proteins.

Mercury (Hg)	IIB	80 and 200.6	+1, +2 [Xe] $4f^{14}5d^{10}6s^2$	Organometallic compounds as methyl, ethyl, phenyl, or dimethyl mercury; inorganic salts mostly chlorides	Used in thermometers, barometers, and batteries; Also used in electrical switches and mercury-vapor lighting products.	Attacks thiol groups, $CONH_2$ and NH_2 of proteins, and amino acids, phosphate group in DNA, cysteine, glutathione, and sulfhydryl (—SH) of GABA. Impairs the activity of glutathione peroxidase by binding to Se. Induces protein precipitation. Mercury appears to modify nuclear antigens that are capable of eliciting reactions from T-cells.	Q3
Nickel (Ni)	VII (iron cobalt transition group)	28 and 58.6	[Ar] $3d^8 4s^2$ +2 +3		Used in electroplating and metal alloys because of its resistance to corrosion; also in nickel–cadmium batteries, as a catalyst and for coins.	Oxidative DNA damage and inhibits DNA repair activity. Hypoxic signaling pathway activation. Induction of cytosine methylation and histone deacetylation. Inhibition of nucleotide excision repair.	

of both the metal center (Hg) and the organic moieties covalently bonded to Hg atom in their molecules. The coordination ability of mercury atom and the cleavage of C–Hg bond is associated with the various pathways that are involved in biochemical processes that cause toxicity by multiple mechanisms. Mercury is one of the few metals due to which there is has been an acute outbreak of toxicity in human populations. In the early 1950s, massive methyl mercury (MeHg) poisoning of residents living around Minamata Bay, a small inlet located on the southwestern coast of Kyushu island, Japan, first raised awareness of the resulting severe neurological disease [Shiraki and Takeuchi, 1971]. Acute MeHg poisoning caused various toxic symptoms such as visual and hearing impairment, ataxia, and psychological disturbances. This led to increased research on the toxic mechanism of action of this metal.

The presence of MeHg in food is linked to its high toxicity. In general, mercury and its properties assist excessive free radical formation, and mercury is implicated as one of major causative factors in the toxic cell damage associated with organomercury compounds. It is known to exert major toxic effects on the central nervous system (CNS). The developing brain, in particular, is vulnerable to methyl mercury toxicity, leading to several neurodevelopmental disorders. The role of glutathione (GSH) and reactive oxygen species (ROS) in MeHg-induced neurotoxicity has been proven. Depletion of GSH increases MeHg accumulation and enhances MeHg-induced oxidative stress; conversely, supplementation with GSH precursor protects against MeHg exposure in vitro. Similarly, Hg-induced modulation of GABA currents is also considered a primary mechanism of action of mercury in which Hg is capable of interacting with the sulfhydryl (—SH) groups on GABA receptors, resulting in increased neurocurrent. Another possible mechanism of action involves altering the phosphorylation of the GABA receptor complex [Fitsanakis and Aschner, 2005]. In summary, neurotoxicity of Hg occurs as a result of a modulation of amino acid metabolism, catabolism, and its regulation. Organic Hg and its implications in cardiovascular diseases (CVD) has received considerable attention recently. This stems predominantly from initial epidemiological findings from Finland that high mercury content in hair was associated with an increased progression of atherosclerosis and risk of CVD. These adverse effects on CVD have been observed at methyl mercury levels lower than those associated with neurotoxicity. The mechanisms by which mercury exerts its effects on CVD are not fully understood. High affinity of Hg for thiol groups and its ability to bind selenium into an insoluble complex reduces antioxidative defenses and promote free radical stress and lipid peroxidation.

Mercury has a high affinity for selenium, and it readily binds selenium to form insoluble mercury selenide complexes. This interaction between mercury and selenium may represent one mechanism through which mercury increases the risk of CVD. Mercury reduces the bioavailability of selenium and impairs the activity of glutathione peroxidase, thus promoting lipid peroxidation and, subsequently, atherosclerosis [Virtanena et al., 2007]. There are at least four different glutathione peroxidases, and all contain selenium in their active site; these enzymes are actively involved in the defense against ROS. Binding to selenium, mercury can reduce the

bioavailability of selenium for incorporation into glutathione peroxidase, thereby inactivating the enzyme. Similar to other trace metals, Hg can act as a catalyst in Fenton-type reactions, which result in the formation of highly reactive hydroxyl free radicals. It has been demonstrated that mercury alters the structural integrity of the mitochondrial inner membrane, resulting in loss of normal cation selectivity. Mercury induces autoimmunity, which is the failure of an organism to recognize its own submolecular levels. The ultimate mechanisms of mercury-induced autoimmunity are not yet understood clearly, although some insights have been gained in relation to T-cell involvement. Mercury appears to modify nuclear antigens that are capable of eliciting reactions from T-cells. There is accumulating research pointing to various mechanisms by which Hg causes toxicity. A variety of studies oriented toward the assessment of biochemical mechanisms responsible for the effects of organomercury compounds (mostly methylmercury) on living organisms are underway. However, the toxicity mechanism is still under debate.

5 MODE OF ACTION: WHAT IS THE FUTURE?

The mode of action (MOA) of trace elements has attracted the attention of researchers and regulators for many years. The literature is abounding with appropriate studies, and MOA-based approaches are becoming important in risk assessments for individual trace elements and in generic risk appraisal guidance documents. In summary, it is seen that most trace metals have the highest common mechanism as DNA perturbations, induction of chromosomal aberrations, and synthesis of new proteins by activation of various kinases and release and activation of capsases, leading to carcinogenicity and cell death as being important aspects of the MOA. ROS and free radical generation and lipid peroxidation is also a common phenomenon seen in all metals either directly or indirectly. In the laboratory, researchers are uncovering new information on the biochemical and cellular changes underlying toxic effects. In the regulatory context, scientists and policymakers are working to harmonize some of the principles and practices that underline toxic effects. All this information will prove its worth only when it is useful in the context of alleviating toxicity after its development apart from prophylactic measures. Microarray analyses reveal global changes in gene expression in response to environmental changes and, thus, are well-suited to provide a detailed picture of trace element MOA [Kawata et al., 2007]. Specifically, these responses are represented by patterns of gene expression signatures, which provide insight into the MOA as well as the general physiological responses of living systems to metal stresses.

DNA microarray technology has helped in uncovering a large amount of MOA information in minimal time and, in addition, has proved to be phenomenal in the area of drug discovery. This is because microarray technology enables high-throughput testing of gene expression to explore a variety of toxicological questions. This, in turn, creates an opportunity to use this information to discover and test novel pathways and therapeutic applications. There is much optimism concerning the use of functional cells derived from stem cell cultures in drug discovery and toxicology.

On the other hand, nanotechnology has helped in drug delivery; elucidation of MOA has helped in developing nanoscale delivery vehicles capable of controlling the release of chemotherapeutic agents directly inside cancer cells targeting the trace metal or the target molecule. Active targeting can be achieved by the functionalization of potential drugs with targets such as antibodies, peptides, nucleic acid, carbohydrates, and small molecules. Transitional structural chemogenomics [Chan et al., 2006] is one of the possible future methods that could prove to be highly useful in trace element therapeutics. This is a method by which one can regulate gene expression, employing ultrasensitive small-molecule drugs targeted toward nucleic acids. Gene expression can be regulated by using chemicals to target transitional changes in the helical conformations of single and double-stranded DNA.

MOA studies have now almost reached the limits of "reductionist" approach. The field has taken a turn to interdisciplinary approach with a systems biology perspective that looks for strength in biology, metabolic engineering, and idiotypic networks. Recently, attention is being focused on harmonizing risk assessment approaches for all toxic endpoints. Harmonization refers to using a biologically dependable approach to risk assessment for all endpoints. As research reveals more MOA information, biological linkages become increasingly clear. MOA throws light on key events in the toxicity pathway specific for an organ, tissue, or cell. These should be conceptualized for toxic effects in various organisms culminating in the ecosphere level. Multiscale models of MOA studies integrate information at different length and time scales, with the horizontal two-way link from the DNA to ecosphere, while amalgamating both the continuum processes (e.g., activation of a specific kinase) and stochastic processes (e.g., the eventual effect on the ecosphere). Multiscale models of MOA will also be taking the human relevance factor, which is a key aspect in the progress of MOA science. Human relevance factor will specifically determine if there is a remedy in the future. It is possible that detailed molecular study of MOA will proceed within the framework of these multiscale models that sum up knowledge of the expansive biological context in an accurate and quantitative manner.

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