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2. Metals

Metals are ubiquitous in the biosphere where they occur as part of the natural background of chemicals to which human beings are constantly being exposed. In fact, one cannot even think of human evolution without considering the great role played by metals in mankind’s development. Despite the benefits derived from the metals, society has to embrace the harsh consequences of metal pollution. Earlier, metal pollution only affected that small portion of the population who were in the local region surrounding the source, however industrial uses of metals and other industrial and domestic processes have introduced substantial amounts of potentially toxic metals into the atmosphere and into aquatic and terrestrial environments. The major problems associated with excessive release of metals into the environment are that metals neither biodegrade, nor are they eliminated by incineration processes. These elements tend to be persistent pollutants, and can accumulate in ecosystems causing detrimental effects and consequences to human health (Rojas et al. 1999).

Of the metal elements, about 40 are considered to be "common metals" however less than 30 have compounds that have been reported to produce toxicity. Metals are probably some of the oldest toxicants known to humans. Health effects such as colic were reported following exposure to lead, arsenic and mercury over 2000 years ago. On the other hand, metals such as cadmium, chromium and nickel belong to the modern era. Each metal has a specific chemical form (speciation) which determines its ability to get incorporated into
the biological systems. Metals seldom interact with biological systems in the elemental form and are usually active in the ionic form. Ionic forms, because of their water solubility, are allowed to enter into biologic processes. They have a tendency to non-discriminately bind to electronegative ligands in the organism and are only slowly excreted. Therefore, they tend to accumulate on continuing exposure. The induction of localised tumors in experimental animals at the site of injection has been demonstrated following parenteral administration of metals themselves, their soluble salts and various insoluble metal compounds (Costa, 1980). During chronic toxicity, the metal distributes itself throughout the body and preferentially binds to the ligand with the highest binding affinity, however in acute toxicity, binding occurs to the first available ligand. Hence route of administration is critical in determining the effects of acute toxicity. Cells with high affinity ligands associated with toxicity, are referred to as target cells. Organometallic compounds are able to pass through biological membranes because of their high degree of lipophilicity. Consequently, membranes such as the blood brain barrier can be permeated and metals are allowed to persist for long periods of time (Winder et al, 1997).

All metals in their ionic form can build up in the body and reach toxic levels. This is true even for iron, which is one of the most important metals to make life possible. Metals in their ionic form (which is quite different to the form they have in our food) can operate as catalysts and create an avalanche of free radicals. One metal ion can catalyse one million molecules. When molecules are broken apart, new free radicals are created and more molecular components are
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destroyed. Finally cells can be destroyed and genetic information is damaged or destroyed. From this point of view, no amount of ionic heavy metals in our body can be regarded as safe.

The carcinogenicity of arsenic, chromium and nickel has been established. Occupational and environmental arsenic exposure is linked to increased lung cancer risk in humans, although experimental studies remain inconclusive. Experimental studies clearly demonstrate the malignant potential of hexavalent (VI) chromium compounds, with solubility being an important determining factor. Epidemiological studies of workers in chromium production and use, link exposure to lung and nasal cancer. Experimental and epidemiological data show that sparingly-soluble nickel compounds and possibly also the soluble compounds are carcinogens linked to lung and nasal cancer in humans. Some experimental and epidemiological studies suggest that lead may be a human carcinogen, but the evidence is inconclusive. Although epidemiological data are less extensive for beryllium and cadmium, the findings in humans of excess cancer risk are supported by the clear demonstration of carcinogenicity in experimental studies. Other metals, including antimony and cobalt, may be human carcinogens, but the experimental and epidemiological data are limited (Magos, 1991; Hayes, 1997).

2.1. Exposure to metals

Humans have been introducing metals into the environment since they first gained knowledge of their many useful properties. Metal pollutants are primarily distributed in the atmosphere, water, soil and sediments. Atmospheric
metal pollution arises mainly from the mining, smelting and refining of metallic ores, the manufacturing and use of metallic products, and the burning of fossil fuels. Atmospheric pollution is often the largest source of water borne metals. Exposure to metals and metal containing compounds is common to many industrial, non-industrial and environmental situations. Among the general population, the exposure to a number of metals is widespread but generally at substantial lower levels than have been found in industry. For most individuals, the greatest cause of metal exposure is due to metal content in food with a smaller additional component coming from air. Some metals are essential for human nutrition, others are found as contaminants in food stuffs. Both essential and toxic metals are present simultaneously in normal human diet (Winder et al 1997; Hayes, 1997; Rojas et al 1999).

Other potential exposure to metals in the non-occupational environment include use of metals as therapeutic material, paediatric mishaps, exposure to industrial wastes and pollution in consumer products such as deodorants, hair dyes and cosmetics etc. Some metals such as arsenic, lead and mercury have a long history, mainly because of their use as poisoning agents, but also for their now classic causes of diseases. Other metals have come to the fore as new technologies and processes require their use (for example Be, Cr, Ni, Ur). The amount of information on health effects varies from metal to metal and of course is dependent on a number of factors including inherent toxicity, availability to workers, the quantity of production and the types of processes in which the metal is employed (Winder et al 1997).
2.2. Cellular uptake of metal carcinogens.

Many of the metals that are potent carcinogens in experimental animals are relatively water insoluble and undergo dissolution slowly at the site of administration. Highly water-soluble metal compounds are not assumed to be very carcinogenic unless they become localised and there is persistent contact with a particular tissue. One reason given for the low carcinogenic activity of water-soluble metal compounds is their rapid excretion from the body through the kidneys. This results in minimal tissue contact time. The water-soluble metal compounds, however, can be carcinogenic if they are released from a reservoir containing metallic material that slowly releases carcinogenic metal ions. An example of this would be a metal implant placed into the body at a site where it remains in constant contact with physiological body fluids that are capable of corroding the metal implant. The corrosion of many alloys releases various ionic salts of the carcinogenic metals such as nickel (2+) and chromium (6+). This slow release of soluble metal salts from the metal implant provides a constant source of tissue exposure to carcinogenic metal ions. It is also possible that water-insoluble carcinogenic metals and their compounds deposited experimentally into a tissue, slowly release water-soluble carcinogenic metal salts; this could occur as a result of a reaction between the animal’s serum and the metal compound. These water soluble metal salts may then be the compounds that enter the cells and cause cancer (Costa, 1980).

Certain experiments conducted with animals using various water-insoluble nickel compounds suggest that 90% of the administered nickel
becomes complexed with ultrafilterable molecules (Webb et al. 1972; Webb and Weinzierl, 1972). These results imply that the insoluble nickel compounds may slowly dissolve in the body fluids and then are taken up by cells only after the nickel is complexed with ultrafilterable molecules. Nickel is known to associate in vivo with amino acids, such as histidine. The tight binding of nickel to amino acids may be the mechanism by which the metal is taken into these cells as an ultrafilterable complex. Insoluble metal compounds when injected locally into experimental animals probably exhibit high carcinogenic activity because their contact with cells results in some cell death, which is followed by differentiation and proliferation of adjacent cells in an attempt to heal the injury. During this process the cells will be actively taking up nutrients from the surrounding environment, which may include water-soluble nickel compounds or ultrafilterable nickel complexes. This proliferation combined with the uptake of the nickel-containing nutrients may result in the nickel becoming distributed within the nucleus. During the DNA replication the nuclear nickel may cause transformation of a normal cell into a cancer cell. This process of cell death and proliferation is essential for the induction of cancer in tissue like muscle, which have a low index of cell proliferation. In tissues where the cell proliferation is naturally very high (e.g. the gastrointestinal tract and pancreas), the contact of the metal with the tissue may result in toxicity, death and subsequent regeneration, resulting in more cell division, and there by increasing the probability of cancer induction by the metal carcinogen (Costa, 1980)

2.3. Molecular mechanisms of metal carcinogenesis
The molecular mechanisms involved in metal carcinogenesis are not well understood, even though a number of metal compounds (e.g. of As, Ni, Cr and Cd) have been identified as potentially carcinogenic to humans based upon epidemiological studies or are known to induce tumors in experimental animals (e.g. Ni, Cr, Cd, Be, Pb) (Costa et al. 1984). The data available suggest that metals cause cancer by a mechanism that is similar to the way in which organic carcinogens induce transformation i.e. by binding to cellular DNA or associated proteins, which then induce mutations. Whether the metal compounds or free metal is converted to an "ultimate" carcinogen is not known, but several different experiments suggest that the ionisation state and the crystalline structure of the metal are important factors in determining its ability to cause cancer. Carcinogenic metals do in fact enter the nuclei preferentially and bind to nucleic acids and proteins within the nuclear structure (Costa, 1980). During the carcinogenic process, metal ions can affect DNA indirectly through oxidative damage, or by effects on DNA polymerase. In cases, where the metals interact directly with DNA, it is difficult to determine the extent of DNA -metal binding because with few exceptions, the co-ordinate covalent binding of metals to the DNA bases is generally unstable to isolation. Additionally, ligand exchange reactions and metal redistribution can occur during the isolation of metal-cellular ligand complexes (Costa, 1991).

Metals have been shown to increase the production of reactive oxygen species (ROS) and/or to inhibit antioxidant activities, directly or indirectly leading to oxidative injuries. Those metals that directly increase the production of
reactive oxygen species include iron, copper, chromium, vanadium and manganese. Iron (Alleman et al. 1985; Halliwell and Gutteridge, 1986) and copper (Chan et al. 1982) are important cofactors in the Fenton reaction to produce reactive oxygen species. Chromium has been shown to facilitate the formation of hydroxyl radical from hydrogen peroxide (Shi and Dalal 1990; Shi et al. 1993). Some metals such as cadmium and nickel increase tissue iron levels, thereby increasing reactive oxygen species generation. Perpetual ROS generation by metals can cause specific molecular changes resulting in the activation or inactivation of transcription factors that may alter gene expression leading to cell proliferation, differentiation and carcinogenesis. ROS signalling is critical for the responses of cytokines, growth factors and activation or inactivation of transcription factor that promote carcinogenesis (Ding et al. 2000).

DNA-binding proteins, known as zinc finger proteins, act as transcription factors by binding specifically to short DNA-sequences and controlling the transcription of a number of genes. Metals such as cobalt, cadmium, copper, nickel and iron substitute for zinc in zinc finger proteins and induce DNA damage and carcinogenesis by generating free radicals. Such metals bound to a DNA-binding protein generating potentially harmful free radicals may be of relevance to the toxicity and carcinogenecity of these metals (Sarkar, 1995). These toxic metals were found to interfere with the zinc in zinc finger DNA repair proteins (Asmuss et al. 2000).

Carcinogenic trace metals vary considerably in chemical properties. They might, therefore be expected to show a spectrum of genotoxic mechanisms
for cell transformation. Prominent as a possible genotoxic mechanism is direct or indirect induction of DNA damage, involving DNA breaks, deletions, rearrangements and base changes. Direct damage might be expected to ensue as a result of metal-DNA adduct formation, metal catalysed DNA-DNA or DNA-protein cross linking or metal interference with replication. Indirect mechanisms could involve the production of active oxygen species as the primary metal reaction. Alternatively carcinogenic metals might through binding to signal transduction intermediates or transcription factors, include error-prone repair of DNA damage. Metal interaction with repair enzymes could also inhibit this function. Either type of activity could result in a synergistic genotoxic effect when combined with or following exposure to other mutagens. Carcinogenic metals may be genotoxic also via clastogenic mechanisms not involving DNA damage but through effects on the mitotic apparatus and process. Aneuploidy would be the expected result in such instances. Also included in the spectrum of carcinogenic metal mechanisms would be protooncogene activation via induction of gene amplification, involving interactions of metal ions with DNA replication intermediate forms and enzyme complexes. Interaction of metal ions with DNA methylases might result in hypo- or hyper-methylation of DNA, there by altering gene expression in epigenetic but heritable (in the somatic cell lineage) fashion (Enger and Kang, 1997).

Specific mechanisms are induced by some metal ions but not others, such as the induction of gene amplification by arsenite and DNA methylation by nickel. Active oxygen appears to play a role in metal induced DNA damage by
cadmium, chromium and nickel ions, at least at higher concentrations. Mechanisms for inhibition of repair are also commonly seen. Effects on the mitotic apparatus are suggested for cadmium but not examined or reported for the other metal ions. Although beryllium ions appear to be both mutagenic and clastogenic, their effects on repair and the possible role of active oxygen species have not been reported. Because these metal ions also affect many signal transduction pathways in such a fashion as to potentially alter cell proliferation and apoptosis, they may well affect cell transformation in a multifactorial fashion and be involved in different ways at several stages of carcinogenesis (Enger and Kang, 1997).

There are two fundamental types of cell death, namely, apoptosis and necrosis. Apoptosis is considered to be the major process responsible for cell death in various physiological events. Necrosis, on the other hand, occurs as a consequence of an injurious environment. It has been well known that metals at their toxic concentrations cause necrosis in a variety of tissues. Recent studies have also demonstrated that metals also induce apoptosis as a mode of toxicity. Biological essential metals such as copper, iron and zinc are associated with apoptosis under conditions of their deficiency; overload of these metals often causes necrosis but not apoptosis. Other metals such as cadmium, chromium, nickel and selenium induce both apoptosis and necrosis when biological systems are exposed to these metals. In general, low dose exposure to these metals most likely causes apoptosis and high doses are associated with necrosis. Exposure to low-dose selenium activates endonucleases such as calcium and magnesium
dependent endonucleases leading to apoptosis (Lu et al 1994). Cadmium induces calcium mobilisation, which would in turn cause activation of endonucleases (ElAzzouri et al 1994). Copper deficiency and toxic metal exposure both generally result in production of reactive free radicals such as reactive oxygen species, which represents another apoptotic mechanism shared by essential metal deficiency and toxic metal exposure. Carcinogenic chromium induces apoptosis by directly interacting with DNA to form DNA-chromium adduct (Blankenship et al 1994). The mechanisms involved in metal induced necrosis include alteration in ion homeostasis, oxidative injury, mitochondrial dysfunction and ATP depletion. Inhibition of DNA, RNA and protein synthesis often occur during metal toxicosis. This inhibitory effect may not be the primary effect of metals, with an exception of carcinogenic metals such as chromium. The ultimate event of metal induced necrosis is plasma membrane damage and cell lysis. Mechanisms for the cadmium induced hepatic necrosis include inhibition of protein synthesis and lipid peroxidation (Dudley et al 1984; Anderson and Anderson, 1988). Chromium has a wide range of effects on living systems. It causes carcinogenesis, apoptosis and necrosis (Wedeen and Qian, 1991; Molyneux and Davies, 1995). Other metals that cause necrosis by overt inhibitory effects include mercury, nickel, vanadium, lead, thallium and platinum. A common primary event induced by metals that cause necrosis is alteration of intracellular calcium homeostasis and subsequent lipid peroxidation. Increase in cellular calcium is often observed during metal toxicities. Two fundamental actions would each produce increases in cellular calcium. First, many metals
directly interact with calcium channels and/or calcium dependent ATPases to alter calcium homeostasis. Second, metals cause oxidative damage to plasma membranes, leading to calcium release from intracellular organelles such as mitochondria and endoplasmic reticula (Enger and Kang, 1997).

Literature on established carcinogens like nickel and chromium is reviewed in details below.

2.3.1. Nickel

Nickel is a very abundant element. In the environment, it is found primarily combined with oxygen (oxides) or sulphur (sulphides). It is found in all soils and is emitted from volcanoes. Nickel is an essential element for at least several animal species. These animal studies associate nickel deprivation with depressed growth, reduced reproductive rates and alterations of serum lipids and glucose. Although there is substantial evidence of an essential status for nickel in animals, a deficiency state in humans has not been clearly defined (Barceloux, 1999).

2.3.1.1. Exposure to nickel

The exposure and toxicity is based on the various classes of nickel compounds and the human activities associated with them. Drinking water and food are the main sources of exposure for the general population with the average American diet containing about 300 micrograms Ni/day. Nickel is not a cumulative toxin in animals or in humans. There is little evidence that nickel compounds accumulate in the food chain (Barceloux, 1999).

Representative exposure data are difficult to obtain. Nickel levels in the environment vary depending upon the location sampled. The average level from
widely scattered atmospheric samples in the US in 1964-1965 was about 340 ng/m\(^3\) (Folk, 1970). Metallic nickel constitutes about 0.03% of the particulate matter suspended in the atmosphere (Sullivan, 1969). Nickel compounds, including sulphides, oxides and carbonyls are also present in the atmosphere. These compounds are thought to enter the atmosphere as a result of the combustion of coal, diesel fuel and fuel oil contaminated with nickel impurities (Natusch et al 1974). Since there is an increasing shortage of petroleum, it is likely that in the future there will be a shift to the use of coal as a primary source of energy. This will most likely result in an increase in the atmospheric levels of a variety of pollutants. Among these pollutants, are the metals and their compounds present in coal as impurities that will be released into the atmosphere with the coal fly ash. Coal burners are equipped with precipitators that attempt to collect the pollutants, but these are rarely 100% efficient. Consequently a variety of carcinogenic metals including Ni, Co, Cr and Cd have been found deposited at high concentrations in the soil collected within a 115 mile radius of the coal burner (Piperno,1975). Nickel and arsenic tend to be associated with small sized particulate material in coal fly ash, but their volatility profiles are such that they are not released as readily into the atmosphere during coal combustion as is cadmium. The metal compounds formed during coal combustion are usually the oxides, but sulphides might also be present. The carcinogenic activity of the various metal compounds formed during coal combustion may differ considerably from that of the free metal.
Warner (1984) reported time weighted average concentration in air in different work places where nickel may be found in high concentrations (above 1 mg/m\(^3\)). These sources are roasting and smelting operations, electrolytic refining and foundry operations. Moderate levels (0.05 to 1.0 mg/m\(^3\)) were found in stainless steel, welding, electroplating and Ni-Cd battery manufacture. The incidence of allergic contact dermatitis through exposure to nickel containing jewellery is relatively high and possibly occur in about 20% of exposed individuals. The sensitising exposure usually comes from the piercing of ears or other body parts.

Nickel is a constituent of over 3000 metal alloys and is used for a huge range of purposes such as coinage (some coins may be 99.8% Ni), stainless steel (which contains about 10% Ni), cooking utensils, corrosion resistant equipment aircraft parts, magnetic equipment, jewellery, rechargeable batteries, medical applications, ceramics and so on (IPCS, 1991). Most of the world’s nickel, is processed from sulphide ores and to a lesser extent, oxides. The organic compound nickel carbonyl is produced in the Mond refining process and because of its volatility (Boiling point of 430°C) and lipid solubility is highly toxic and carcinogenic.

In humans, adverse effects of water soluble nickel compounds occur after skin contact (causing contact dermatitis) and after inhalation (which causes respiratory tract irritation and asthma) in workers such as electroplaters. Human exposure to inorganic, water insoluble nickel compounds usually occurs through
inhalation of fumes or dust, which are associated with cancers of the respiratory tract among workers in nickel refineries (Winder et al. 1997).

Wide variations have been reported in body nickel levels. Further because of decreased emissions and better analytical methods, exposures are decreasing, suggesting that earlier data are not representative of contemporary conditions. Background levels of nickel in human blood are less than 0.5 \( \mu \text{g/l} \) and about 2 to 3 \( \mu \text{g/l} \) in urine. Both blood and urine are used to monitor workers. Elevated urine nickel levels are shown in some nickel workers with the highest being in nickel refinery workers (with a mean of over 200 \( \mu \text{g/l} \)) (Bernacki et al. 1974). Occupational groups had urinary levels below 20 \( \mu \text{g/l} \). Workers exposed to nickel compounds have elevated levels in both urine and plasma with plasma being suggested as the more reliable measure (Lauwerys and Hoet, 1993; Elinder et al. 1994). At the present time there is no clear relationship between health risks and levels in body fluids, except for over exposure to nickel carboxyls. A potentially complicating factor is the exposure to nickel compounds of low solubility. Slow clearance of these compounds may be responsible for elevated level in workers for some years after cessation of exposure. It is also these low solubility nickel compounds (like nickel subsulphide) that are linked more to lung cancer (Winder et al. 1997).

Biologically nickel is considered to be a 'new' trace element with tissue concentration in ng/g amounts and is involved in facilitating the intestinal absorption of ferric iron. Findings that nickel absorption is significantly increased in iron deficient rats suggest that nickel ions use the iron-transport system
located in the proximal part of the small intestine. Nickel is apparently transported across the mucosal epithelium via an active energy dependent process rather than by simple diffusion. Dietary substances such as phytate and EDTA have been reported to decrease nickel absorption. Consistent symptoms of nickel deficiency in animals are ultrastructural abnormalities in the liver, depressed growth and iron deficiency. However, in humans, no effects of deficiency are known (Teitz, 1986).

Limited studies indicate that human dietary intakes of nickel range from 170-700 µg/d. A human dietary requirement for nickel has been estimated to be 50 µg/kg (Nielsen, 1982). Diets based on foods of animal origin are likely to be low in nickel. Rich sources of nickel include chocolate, nuts, peas, beans and grains. Smart and Sherlock (1987) reported that nickel levels in nuts (5ppm) and cocoa (10ppm) are high compared with a nickel concentration in most foods below 0.5 ppm. Nickel concentration up to 6.5 µg/g have been reported in cereal food stuffs, levels up to 2.6 µg/g in vegetables, 1.7 µg/g in fish and 7.6 µg/g in tea. Concentration of nickel in water samples can range from 3 to 26 µg/l (CMBEEP, 1975). Nickel is present in cigarettes also. According to Sunderman and Sunderman (1961), six brands of American cigarettes contained an average of between 1.59 and 3.07 µg nickel per cigarette and 20% of this is inhaled with the smoke. Since the average smoker will inhale about 20 cigarettes per day, 0.8 mg of nickel will be accumulated in one year (Lewis et al 1972)

The amounts of nickel concentration in the wine samples analysed by Teisseder et al (1996) ranged from 5.4 to 87.9 µg/l in red wine, from 7.5 to 74.4
μg/l in white wine, from 19.5 to 24.6 μg/l in rose wine and from 8.9 to 26.9 μg/l in champagne. Nickel content in grapes ranged from 4.2 to 94.0 μg/kg for the 12 different varieties.

In addition to atmospheric, water and food pollution with nickel, this metal is also used in wide variety of surgical and dental implants. Nickel is present in stainless steel and the nickel wires of suture materials (Wu et al 1967), nickel-chrome metallic mesh for nasal prosthesis (Narita, 1966), stainless steel, heart wire prosthesis (Sawyer et al 1967), nickel containing intrauterine contraceptive devices (Chang et al 1970; Kesseru et al 1974; Ober et al 1970), Ni-Cd batteries for implantable cardiac pacemakers (Hershberg et al, 1965) and nickel alloys for orthopaedic implants and dental fillings (Barranco and Soloman, 1972; Dube and Fisher 1972; Harty and Leggett, 1972; Mckenzie et al 1967). Cadmium and chromium are also sometimes implanted with nickel into humans. Stainless steel #316, for example contains about 8% nickel and 18% chromium. This alloy is often used in human implantation devices. Evidence exists that these implanted alloys containing nickel do in fact cause cancer in experimental animals and humans. Mitchell et al (1960) implanted 4 pellets of nickel-gallium dental filling material (60% Ni-40% Ga) subdermally at a number of sites into wistar rats and found that 9 of 10 rats developed sarcomas. Only 5 of 10 rats developed sarcomas that received pure nickel implants. Humans have developed sarcomas following implantation of stainless steel material that contained nickel and chromium (Dube and Fisher, 1972; McDougall, 1956). The corrosion products, especially nickel and chromium were thought to be responsible for the
hemangioendothelium in the tibia of a patient who received a steel plate implantation (Dube and Fisher, 1972)

2.3.1.2. Nickel carcinogenesis

Nickel compounds represent well established human carcinogens based upon epidemiological studies. Laboratory experiments have shown nickel to induce tumours in animals (IARC 1976; Gilman, 1965) and to cause neoplastic transformation in tissue culture systems (Casto et al 1976). Nickel compounds are both primary and secondary carcinogens (Luckey and Venugopal, 1977). However, determining the mechanism of carcinogenicity has proven difficult, most probably because nickel binds cellular compounds weakly and reversibly (Klein et al 1991). Possible mechanisms of nickel carcinogenesis are cellular uptake and intracellular translocation of nickel, morphological transformation of cells by nickel compounds, chromosomal damage, DNA strand breaks and DNA protein complexes produced by nickel compounds, mutagenic effects of nickel, influence of nickel on the helical transition of B-DNA to Z-DNA, nickel induced infidelity of DNA synthesis, free radicals and lipid peroxidation induced by nickel exposures, nickel inhibition of DNA repair, nickel as tumor promoter, nickel inhibition of natural killer (NK) cell activity, manganese and magnesium antagonism of nickel carcinogenesis and speculation that Ni$^{2+}$ might replace Zn$^{2+}$ in finger loop domains of transforming proteins.

The relationship between structure and activity for the carcinogenic response of nickel compounds is of predominant importance for those metal compounds of low water solubility since the numerous metal compounds readily
dissolve from only a limited number of extra cellular cationic species. A few metal compounds such as nickel carbonyl are very lipid soluble and readily enter the cell, producing toxic and carcinogenic effects at relatively low concentrations (Sunderman, 1978). Experimental animal carcinogenesis studies have demonstrated that crystalline nickel sulphide particles may in most cases induce a 100% incidence of cancers at any one of a variety of administration sites while similar treatment with amorphous nickel sulphide particles which have low water solubility generally does not result in the induction of tumors (Sunderman, 1978). Thus, it appears that certain particulate nickel compounds have very potent carcinogenic activity while others possess virtually no oncogenic activity. Water soluble nickel compounds as a class are consistently low in toxic and carcinogenic potential.

The relative toxicity and carcinogenicity of nickel sulphate hexahydrate (NiSO₄·6H₂O), nickel subsulphide (Ni₃S₂) and nickel oxide (NiO) were studied in F344/N rats and B6C3F1 mice after inhalation exposure for 6h/day and 5 days/week for 2 years. Nickel subsulfide (0.15 and 1 mg/m³) and nickel oxide (1.25 and 2.5 mg/m³) caused an exposure related increased incidence of alveolar/bronchiolar neoplasms and adrenal medulla neoplasms in male and female rats. Nickel oxide caused an equivocal exposure related increase in alveolar/bronchiolar neoplasms in female mice. No exposure related neoplastic responses occurred in rats or mice exposed to nickel sulphate or mice exposed to nickel subsulfide. These findings are consistent with the results from other studies, which show that nickel subsulfide and nickel oxide reach the nucleus in
greater amounts than do water soluble nickel compounds such as nickel sulphate (Dunnick et al 1995).

2.3.1.2.1. Bioavailability of nickel

The carcinogenic potencies of different compounds appear to be related to bioavailability of nickel (presumably Ni\(^{2+}\)) to critical intracellular sites (Costa and Heck, 1984). Crystalline nickel sulphide compounds, which are potent in inducing tumors in animals, also transformed cells in tissue culture. In contrast, the amorphous nickel sulphide particles, which did not induce tumors in animals were not active in transforming cultured cells. In tissue culture studies, crystalline nickel subsulfide compounds were actively phagocytized by cultured fibroblasts undergoing transformation but non-carcinogenic amorphous nickel sulphide particles were not (Costa and Mallenhauer, 1980). Based upon studies, it was proposed that cells phagocytising these particles in vivo included not only professional phagocytes such as macrophages but also nonmacrophage cancer forming target cells, via the process referred to as facultative phagocytosis (Costa and Heck, 1984). Phagocytosis of nickel particle would deliver large quantities of metal into the cells and may explain why certain less water soluble nickel compounds are more carcinogenic than freely soluble compounds. It was found that surface properties of these particles were important to their ability to gain entry into cells. By zeta potential measurements, amorphous nickel sulphide particles were found to have a positive surface charge, whereas crystalline nickel sulphide compounds have a more negative surface charge (Abbrachio et al 1981, 1982).
Another situation that results in differential uptake of particles is the release of nickel ions from parent particles and their effects on phagocytic activity (Costa and Heck, 1983). Soluble nickel ions have been shown to inhibit phagocytosis. Consequently weakly carcinogenic amorphous nickel sulphide particles, which were dissolved at slightly greater rate than the crystalline nickel sulphide particles, might release more biologically available nickel ions that inhibit the phagocytic process (Costa and Heck, 1983).

2.3.1.2.2. Intracellular mechanism for the induction of cellular transformation by phagocytized nickel sulphide (NiS) particles

Following phagocytosis of the NiS particles, the particles undergo cytoplasmic dissolution. Video intensification microscopy studies demonstrated that the uptake of crystalline NiS particles occurs largely in areas of cell ruffling. Negatively charged crystalline NiS particles appear to bind reversibly to the cell membrane while positively charged amorphous NiS particles will in some cases detach after binding. Perhaps the binding of the negatively charged particles cause a change in cell membrane architecture which results in particle endocytosis. Video time lapse microscopy studies have shown that following phagocytosis, particles move about in cytoplasm by saltatory motion whereas lysosomes have been observed to interact repeatedly with particles (Evans et al 1982). Additional studies have demonstrated that the mean particle half life in the cytoplasm of the cell is about 2-3 days while particles residing in the extracellular medium dissolve less rapidly. Particle dissolution is extremely important for nickel delivery into the nucleus. Since electron microscopy studies have shown that the
NiS particles cannot cross the intact nuclear membrane boundary, phagocytized NiS particles tend to aggregate in the perinuclear region where dissolved nickel may have ready access to the nucleus or may interact with macromolecules, exiting the nucleus. Since most phagocytized NiS particles are contained in a vacuole the internalised particle itself probably has few intracellular effects (Costa and Mollenhauer, 1980). Ionic nickel is released from the particle and produces a variety of effects upon DNA. Nickel ions have been shown to decrease the fidelity of DNA synthesis (Sirover and Loeb, 1976), promote crosslinking of DNA to protein (Ciccarelli et al., 1981), induce single strand breaks (Robison and Costa, 1982) and cause sister chromatid exchanges (Saxholm et al., 1981). Ionic nickel may interact directly with bases or phosphate moieties of DNA, or it may be competing for natural binding sites (Zn^{2+}) involved in DNA homeostasis. These effects could alter DNA structure and function, resulting in the induction of mutations which may lead to transformation and carcinogenesis. Alternatively, the metal ion or the process of particle phagocytosis itself may enhance the production of oxygen radical intermediates resulting in the formation of reactive malonaldehyde which could promote DNA protein crosslinking with resultant mutagenic effects (Costa and Heck, 1982).

Protection against the toxic and carcinogenic effects of heavy metals probably arises from their ability to induce and interact with metallothionein, a 6800 mol. wt. protein consisting of 30% cysteine. This protein has an extremely high affinity for a variety of toxic metals and is a natural chelator of zinc. Certain metal ions such as cadmium are potent inducers of metallothionein but it remain
to be established whether the metal ion itself or free Zn\(^{2+}\) displaced by Cd\(^{2+}\) from its native metallothionein binding site causes induction of the protein. Activity of metals to induce metallothionein correlates with their ability to bind to the protein, the more cytotoxic metals are the most potent inducers of metallothionein. Nickel is potently carcinogenic, not very cytotoxic and is a poor inducer of metallothionein. This suggests that metallothionein may play an important role in protecting the cell from the genotoxicity of metal ions and since nickel does not potently induce metallothionein, its carcinogenic effects may be greatly enhanced (Costa and Heck, 1982).

The cytoplasmic dissolution of even a small phagocytized NiS particle may result in intracellular nickel levels sufficiently high to exceed cellular protective mechanisms. A theoretical calculation reveals that total dissolution of a 4.0 \(\mu\)m particle within the cell could yield a 4.75 M nickel concentration. Even partial dissolution of such a particle in close apposition to the nucleus may result in nickel ion interaction with DNA at any structural and functional level. The exquisite sensitivity of DNA to nickel effects has been demonstrated with the detection of single strand DNA breaks within 2h following exposure of cells \textit{in vitro} to 1-10 \(\mu\)g ml\(^{-1}\) \(\text{NiCl}_2\), a level at which no overt signs of cytotoxicity are apparent (Robison and Costa, 1982). Thus cells which phagocytize crystalline nickel sulphide particles and survive the resulting high intracellular Ni\(^{2+}\) levels are likely to experience altered genetic function. The efficient delivery of crystalline nickel sulphides into mammalian cells by endocytosis may therefore
account for the results of animal studies which show that these compounds are among the most efficient and potent carcinogens (Sunderman, 1978).

2.3.1.2.3. Nickel induced oxidative damage

A possible mode of action for soluble nickel compounds to cause DNA damage is by inducing the Fenton/Haber Weiss generation of hydroxyl radicals (Klein et al. 1991). Oxygen derived species such as the superoxide radical (O$_2^-$), H$_2$O$_2$ and the hydroxyl radical (OH*) have been implicated in the etiology of many human diseases including cancer (Halliwell and Gutteridge, 1989). Thus an increased production of oxygen derived species within cells frequently leads to DNA damage by a variety of mechanisms (Halliwell and Aruoma, 1991) and such species can probably both initiate and promote cancer (Halliwell and Aruoma, 1991; Cerutti, 1985). However, neither O$_2^-$ nor H$_2$O$_2$ reacts chemically with DNA unless metal ions are present in the system (Halliwell and Aruoma, 1991; Aruoma et al. 1989, Blakely et al. 1990). By contrast highly reactive OH* attacks all constituents of DNA producing a multiplicity of chemical changes in the deoxyriboses, pyrimidines and purines (Von Sonntag, 1987). DNA Protein crosslinks also result from OH* attack upon nucleoprotein (Oleinick et al. 1987).

A number of transition metal ions can catalyse OH* formation from O$_2^-$ and H$_2$O$_2$ and thus can induce DNA damage in the presence of these oxygen derived species. They include iron ions and copper ions (Aruoma et al. 1989; Halliwell and Gutteridge, 1990; Aruoma et al. 1991; Dizdaroglu et al. 1991). There is evidence for binding of Ni(II) to cell nuclei (Ciccarelli and Wetterhahn, 1982;
Kasprzak and Poirier, 1985) and for induction by Ni(II) DNA strand breaks and DNA protein cross links (Ciccarelli and Wetterhahn, 1982; Ciccarelli et al, 1981; Robison et al, 1982; Patierno et al, 1985; Kasprzak and Bare, 1989). However, Ni(II) alone causes no damage to isolated DNA (Kawanishi et al, 1989) and the relatively weak interactions between Ni(II) and DNA are unlikely to be responsible for genotoxic effects in cells exposed to nickel (Kasprzak and Bare, 1989).

Thus it has been proposed that Ni(II) reacts with endogenous H$_2$O$_2$ in cells to form OH$^\cdot$ which causes DNA damage (Kawanishi et al, 1989; Kasprzak and Hernandez, 1989). Recent in vitro studies have indicated the formation of OH$^\cdot$ in reactions of Ni(II) and Ni(II)-peptide complexes with H$_2$O$_2$ (Kawanishi et al, 1989; Kasprzak and Hernandez, 1989; Inoue and Kawanishi, 1989; Torreilles and Guerin, 1990). When OH$^\cdot$ is generated by reaction of H$_2$O$_2$ with transition metal ions bound to the DNA, it is often difficult to completely protect the DNA from OH$^\cdot$ attack by adding OH$^\cdot$ scavengers because of the possible "site specific" generation of OH$^\cdot$ (Goldstein and Czapski, 1986; Stoewe and Prutz, 1987; Dizdaroglu et al, 1991; Aruoma et al, 1991; Halliwell and Gutteridge, 1990). Liver iron levels have been found to increase significantly following nickel administration (Ather et al, 1987). Also the characteristic pattern of DNA oxidative products following hydroxyl radical attack has been reported in rats and mice following nickel acetate administration (Kasprzak, 1991).

Iron can initiate lipid peroxidation by catalysing formation of hydroxyl radicals from H$_2$O$_2$. The hydroxyl radical then will abstract a hydrogen atom from
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an unsaturated lipid. The lipid radical then reacts with molecular oxygen to form a lipid peroxide. Iron can further potentiate peroxidative damage by catalysing the decomposition of lipid hydroperoxides to highly cytotoxic aldehydes (Younas and Siegers, 1985). Furthermore, nickel itself may catalyse hydroxyl radical generation via the Haber-Weiss reaction when it is stabilised by chelation to peptides containing a glycyl-glycyl- L-histidine sequence (Torreilles and Guerin, 1990).

Carcinogenesis may be initiated directly by the hydroxyl radical or via lipid peroxidation (LPO). Since malondialdehyde and other lipid peroxidation products are mutagenic and therefore may be carcinogenic (Ames et al 1982; Basu and Marnett, 1983). Lipid peroxidation has been shown to occur in vivo, in liver, kidney and lung, following exposure to NiCl₂ (Sunderman et al 1985, Donskoy et al 1986). Inhibition of catalase, peroxidase and superoxide dismutase might constitute another indirect way by which nickel promotes oxidative damage to cells, this time through H₂O₂ which is a genotoxic and mutagenic agent (Ames, 1983). Lipid peroxidation and alterations in cellular systems protection against oxidative damage were determined in the liver, kidney and skeletal muscle of male F 344/N rats, 1h to 3 days after a single intraperitoneal (i.p.) injection of 107mmol nickel(II) acetate per kg body weight by Misra et al 1990. At 3h, when tissue nickel concentrations were highest, increased LPO (by 43%), increased renal iron (by 24%) decreased catalase and glutathione peroxidase (GPx) activities (both by 15%), decreased glutathione (GSH) concentration (by 20%), decreased glutathione reductase (GR) activity and increased glutathione-s-
transferase (GST) activities (by 44%) were observed in kidney. The activity of superoxide dismutase (SOD) and gamma-glutamyl transferase as well as copper concentration, were not affected. In the liver, nickel effects included increased LPO (by 30%), decreased catalase and GPx activities (both by 15%), decreased GSH level (by 33%) and decreased GR activity (by 35%). SOD, copper and iron remain unchanged. In muscle nickel treatment decreased copper content (by 30%) with no effects on other parameters. Nickel treatment caused profound cell damage as indicated by increased LPO in liver and kidney and leakage of intracellular enzymes, alanine transaminase and aspartate transaminase to the blood (Misra et al. 1990).

Torrellas and Guerin (1990) have shown nickel(II) as a temporary catalyst for hydroxyl radical generation. Peptides containing the glycyl-glycyl-L-histidyl sequence trigger nickel dependent production of oxygen radicals which can damage proteins, cause a rapid loss of tryptophan and a significant production of bityrosine and also induce peroxidation of polyunsaturated fatty acids. During the reaction, the histidine in the peptide is selectively damaged and breakdown of the peptide switches off hydroxyl radical production. The effect of nickel on SOD activity as well as on rate of hydroxydopamine oxidation was studied in vitro by Shainkin-Kestenbaun et al. (1991). Nickel strongly inhibits SOD activity. The degree of inhibition is directly proportional to the nickel concentration. Autoxidation of 6-hydroxydopamine was increased by nickel concentrations higher than 15 micrograms/ml. The combination of excessive oxygen free radical production and inhibition of their elimination by inhibition of SOD activity may
contribute to the nickel toxicity that has been reported in industrial accidents as well as to the high incidence of cancer occurring in nickel workers.

The relationship between nickel-induced hepatic lipid peroxidation and the concentration of nickel and trace elements was investigated in male mice (Chen et al 1998). Hepatic LPO and the concentration of Ni, Fe, Cu and Zn in the liver were enhanced after an i.p. injection of NiCl2. LPO increased significantly in a dose-dependent manner.

In time course studies mice were administered NiCl2 (170μmol Ni/kg) and killed at intervals of 6, 12, 24 and 48h after injection. Both LPO and the accumulation of Ni, Fe, Cu and Zn in the liver showed a significantly positive time-course relationship after NiCl2 injection.

2.3.1.2.4. Effect of nickel on DNA polymerase activity and fidelity

The effects of Ni2+ ions on DNA polymerase activity and the fidelity of DNA replication in vitro were investigated by Snow et al (1993). The effect of Ni2+ on different DNA polymerases is quite variable. The amount of enzyme inhibition and degree of alteration in replication fidelity induced by Ni2+ are dependent both on the polymerase and its associated 3'-5'- exonuclease activity. Some polymerases such as E Coli DNA polymerase I and human DNA, poly α, can utilise Ni2+ as a weak substitute for Mg2+ during DNA replication. Other polymerases are very sensitive to inhibition by Ni2+ and the IC50 can vary by an order of magnitude. T4 polymerase is relatively insensitive to inhibition by Ni2+, although the sensitivity is enhanced in the absence of added Mg2+ and nickel
preferentially inhibits 3'-5'-exonuclease function of T4 DNA polymerase. The fidelity and processing of DNA polymerase may be either increased or decreased by nickel ions in a polymerase dependent manner. The inhibition of DNA polymerase activity and altered replication fidelity may contribute significantly to nickel induced mutagenesis and genotoxicity in vivo. The effect of nickel on DNA replication was studied in a reconstituted in vitro system using a HeLa cell as a source of polymerase activity on template of activated calf thymus DNA (Christie and Tummolo 1998). Nickel has an initial stimulatory effect that is followed by an overall inhibition of the incorporation of DNA precursors. These results suggest that Ni(II), similar to Mg(II) may have more than one binding site but that binding of Ni(II) to replication proteins may significantly alter the timing of events in DNA synthesis.

2.3.1.2.4. Nickel Immunotoxicology

Within the last few decades experimental data have shown that low-level exposure to certain metals induce subtle changes within a host, including altered immunologic competence. Although effects of metals are dependent upon such variations as host species and exposure parameters, including route, dose and duration, the conclusion reached in most immunotoxicological studies is that heavy metals act to suppress immunocompetence. The most consistent finding in experimental and epidemiological studies evaluating the effect of metals on immune functions is a decreased host resistance to infectious agents. Immunotoxicity of metals may occur via direct effects on specific components of the immune system or alternatively via inhibition of immunoregulation, which can
result in immunosuppression, hypersensitivity reaction or autoimmune disorders. It has been postulated that metal toxicity may, at least in part, be due to autoimmunity, since an autoimmune disorder exists for all the major target organs affected by heavy metals (Zelikoff and Cohen, 1997).

Activation of vascular endothelium is a key event in the initial phase of an inflammatory reaction e.g. to contact allergens. Effect of contact sensitizers, NiCl₂ on endothelial expression of intercellular adhesion molecule-1, vascular cell adhesion molecule and endothelial leukocyte adhesion molecule-1 was studied by Goebeler et al (1993). Nickel chloride was found to upregulate these adhesion molecules on human umbilical vein endothelial cells in a dose-and time-dependent manner. Induction of adhesion molecules by NiCl₂ was found to depend on de novo mRNA and protein synthesis. Allergens such as NiCl₂ and CoCl₂ are capable of directly activating endothelial cells which is an important step in evolving inflammatory reactions and may thus be of relevance for the pathogenesis of contact hypersensitivity (Goebeler et al 1993).

Jaramillo and Sonnenfeld (1993) studied the effect of nickel sulphide on induction of interleukin-1 and phagocytic activity. Rat peritoneal macrophages were treated with crystalline nickel sulphide and then tested for interleukin-1(IL-1) production and phagocytosis of IgG-opsonized sheep red blood cells. Pretreatment of peritoneal macrophages with crystalline NiS did not affect their ability to produce IL-1. In contrast, pretreatment of peritoneal macrophages with crystalline NiS inhibited the phagocytic activity of the peritoneal macrophage. This inhibitory activity was due to an effect on the internalisation step of the
phagocytic process. The results described in this study suggest that crystalline NiS has a potent immunomodulatory effect on macrophage phagocytic activity (Jaramillo and Sonnenfeld, 1993). Nickel hydroxy carbonate increases tumor necrosis factor alpha and interleukin 6 secretion by alveolar macrophages (Arsalane et al. 1994). The ability of nickel hydroxy carbonate to activate alveolar macrophages and to release increased amounts of pro-inflammatory mediators may be responsible, at least partly, for inflammation and pneumotoxicity associated with nickel exposure.

Testa et al. (1995) studied neutrophil activation in nickel sensitised subjects. A reduction of B cell polyclonal response, mixed lymphocyte reaction, T-lymphocyte proliferation and natural killer cytotoxicity was evident following nickel salt exposure. Under different experimental conditions, respiratory burst induction and myeloperoxidase release by polymorphonuclear (PMN) cells in a group of individuals exhibiting nickel hypersensitivity were investigated. Results provide evidence that nickel allergic individuals displayed a significant increase of superoxide anion (O$_2^-$) generation by suspended PMN in comparison with similar cell suspensions from healthy donors. PMN from nickel-sensitised donors exhibited a significant enhancement of H$_2$O$_2$ and myeloperoxidase release. The results imply the occurrence of neutrophil activation in nickel hypersensitivity, which in turn may be responsible for the low frequency of life threatening infections in these subjects.

Goutet et al. (2000) investigated the in vivo effect of nickel sulphate on the pulmonary non-specific immune defences. It was found that natural killer cell
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activity and alpha-tumor necrosis factor (TNF-alpha) secretion are sensitive targets for instilled nickel sulphate in Wistar rats.

2.3.1.2.5. Nickel induced DNA damage

Complete chemical carcinogens, including the carcinogenic nickel compounds, which directly induce transformation in cell culture and tumors at virtually any site of administration in animals, also exhibit some DNA damaging activity. Nickel induced DNA damage in vivo has been studied following nickel carbonate administration to female Sprague-Dawley rats (Ciccarelli and Wetterhahn, 1984). Nickel carbonate was found to induce single strand breaks in kidney and lung but not in liver. It was also found to induce the formation of DNA protein cross links in kidney. Using mammalian cultured cells, both water soluble nickel salts such as nickel chloride and relatively water insoluble salts including crystalline nickel sulphide, which were phagocytized by these cells, induce single strand breaks (Robison et al 1982). The formation of DNA protein cross links in cultured cells by nickel compounds exhibits a very narrow dose-response range (Patierno and Costa, 1985; Patierno et al 1985). In several studies, DNA protein cross linking was not detectable in non proliferating cells whereas proliferating cells exhibited protein DNA cross linking and strand breaks (Patierno and Costa, 1985; Patierno et al 1985). However, cells synchronized and treated with nickel during various phases of the cell cycle exhibited substantially higher amounts of strand breaks and DNA-protein cross links compared with the cells in other phases of the cell cycle (Patierno et al 1985).
Various nickel salts (chloride, sulphate, subsulfide, carbonate) have been reported to induce B-Z conformational transition of double-stranded poly (dG-dC) which is often stabilised by supercoiling and DNA methylation. As shown by ultraviolet absorption and circular dichroism, in all cases, nickel adopts the hexacoordinated ionic form \([\text{Ni(H}_2\text{O}_6)]^{2+}\) and induces B-Z transition of the nucleic acids at submillimolar concentration (typically 0.4 mM) (Behe and Felsenfeld, 1981; Bourtayre et al 1984).

DNA-protein cross links (DPCs) are thought to be important genotoxic lesions induced by environmental agents and carcinogens (Smith, 1962; Fornace and Little, 1977; Cosma et al 1988; Patierno and Costa 1985; Wedrychowski et al 1985). These lesions, unlike the strand breaks and other DNA lesions that are readily repaired, are relatively persistent in the cells (Oleinick et al 1987; Sugiyama et al 1986). Due to a poor repair capacity, DNA-protein complexes may be present during DNA replication and possibly cause a loss of important genetic material that may, for example, inactivate tumor suppresser genes (Costa 1991; DeFloro and Wetterhahn, 1989). Many studies with agents such as potassium chromate, calcium chromate, lead chromate, nickel chloride, nickel carbonate and others have revealed that they can induce DPCs. (Sugiyama et al 1986; Klein et al 1991; Kasprzak, 1991; Lin et al 1992; Coogan et al 1988, 1991). Nickel was thought to form stable protein-nickel-DNA complexes and a strong interaction between nickel and amino terminal residues and imidazole group of histidine residues was demonstrated (Coogan et al 1988). On the other hand increasing evidence suggests that nickel may generate reactive oxygen...
species, which may indirectly mediate DNA damage, protein oxidation and DPC formation (Klein et al. 1991; Kasprzak, 1991). Ciccarelli et al. (1981) had detected DPCs in kidney nuclei from nickel carbonate treated rats. DPCs were also found in WBC and lungs of rats treated with NiCl₂ and there was a dose dependent relationship between nickel exposure and DPCs in both tissues (Lei et al. 1995). It is indicated that nickel compounds can generate DNA or protein damages by formation of DPCs, which suggested that DPCs can be used as a biomarker for quantitative NiCl₂ exposure and genetic lesions (Lei et al. 1995).

A unique feature of nickel carcinogenesis is that nickel may induce DNA condensation and hypermethylation in certain nickel-responsive genomic regions. It is an indirect effect, whereby nickel may first induce chromatin condensation and is much more effective than Mg²⁺ in this regard (Weith, 1983; Borochov et al. 1984). DNA condensation could be seeded directly by the nickel ions or by their interactions with histones which are usually concentrated in condensed, nontranscribed DNA.

Nickel exerts its clastogenic and sister chromatid exchange effects with preferential reactivity towards condensed dense regions of heterochromatin (Christie and Katsifis, 1990) as confirmed in studies of Chinese hamster and mouse cells (Cedar and Razin, 1990). The concentrated heterochromatin of the long arm of the hamster X-chromosome is shown to be a hot spot for both NiCl₂- and NiS- induced decondensation and damage, including frequent deletions, (Conway and Costa, 1989). The X-chromosome has also been identified to bear
an epigenetically regulated senescence gene that can be silenced by carcinogenic nickel compounds (Klein et al 1991).

The silencing of tumor suppresser genes, whether by physical deletion yielding allelic loss or by genetic silencing involving DNA methylation (Laird and Jaenisch, 1994) is now recognised as an important feature of the carcinogenic process. Highly carcinogenic insoluble nickel compounds induce an increase in chromatin condensation. As a result, cancer related genes, that are actively expressed in euchromatin, may become condensed into heterochromatin and are stabilized by subsequent DNA methylation. Actively expressed genes have less cytosine methylation in their promoters whereas hypermethylation of cytosine in promoters is a characteristic of inactive genes. Thus nickel induced increases in heterochromatin condensation and hypermethylation of DNA may cause inherited inactivation of critical tumor suppresser or senescence genes. Loss of cellular senescence and the acquisition of cellular immortality is an extremely important step in nickel-induced human carcinogenesis (Costa, 1995; Lee et al 1995; Costa, 1996).

promotes microsatellite mutations in human cancer cell lines raises the possibility that genetic instability may be a mechanism involved in nuckel carcinogenesis (Zinolddinyl et al 2000).

Carcinogenic nickel compounds cause silencing of reporter genes when they are located near telomere or heterochromatin in either yeast or mammalian cells. Due to their abundance, histones are likely targets for nickel ions among nuclear macromolecules (Broday et al 2000; Bal et al 2000). At nontoxic levels, nickel decreased the levels of histone H4 acetylation in vivo in both yeast and mammalian cells, affecting only lysine12 in mammalian cells and all of the four lysine residues in yeast. In yeast, lysine 12 and 16 were more greatly affected than lysine 5 and 8. A histidine Ni2+ anchoring site is present at position 18 from the NH2 – terminal tail of H4 (Broday et al 2000).

Nickel(II) has been shown to interfere with the repair of different types of deoxyribonucleic acid lesions. Concerning the repair of oxidative DNA damage induced by visible light, non-cytotoxic concentrations of nickel(II) cause a complete repair inhibition of DNA base modifications like 7,8-dihydro-8-oxoguanine and of DNA strand breaks. Nickel has been shown to reduce the incision and the ligation frequency and interfere with the DNA-protein interactions involved in the damage recognition after UV-irradiation. Since the repair of DNA damage is essential for the prevention of cancer, its inhibition may account for the carcinogenic action of the respective metal compounds (Hartwig et al 1996).

Shiao et al (1998) studied the effects of nickel(II) acetate on cell cycle, apoptosis and p53 expression in order to unveil the elements of early cellular
responses to the metal. It was suggested that nickel(II) modulates cellular response through effectors involved in both G₂/M arrest and apoptosis regulatory pathways. The proportions of cells arrested at G₂/M phase or undergoing apoptosis depends directly on nickel(II) concentration. High concentration of nickel(II) appears to up-regulate protein(s) other than the common form of p53 protein.

Epidemiological evidence suggests that certain exposures to metals may increase the risk of cancer in the progeny. This effect may be associated with promutagenic damage to sperm DNA. The latter is packed with protamines which might sequester carcinogenic metals and moderate the damage. Human protamine P₂ has an amino acid motif at its N-terminus that can serve as a heavy metal trap especially for nickel(II) (Liang et al 1999).

Salnikow et al (1999) have demonstrated that the activity of hypoxia-inducible transcription factor 1 (HIF-1) responsive promoters was increased in nickel-transformed rodent cells, resulting in the increased ratio between HIF-1 and p53-stimulated transcription. HIF-1 mediates the induction of genes required by cells to survive hypoxia (Salnikow et al 2000). The Cap43 gene, which expressed a 3.0-kb mRNA encoding a Mr 43,000 protein, was found to be induced by nickel in all tested human and rodent cell lines in vitro and also in several rat organs after oral exposure to NiCl₂. Induction of Cap43 is dependent upon HIF-1 because only HIF-1 proficient cells induces Cap43 when exposed to either hypoxia or nickel. Induction of this gene is nickel specific as other tested metal compounds failed to induce Cap43 expression. Primary signal for Cap 43
induction is an elevation of free intracellular Ca\(^{2+}\) caused by Ni\(^{2+}\) exposure. It is suggested that induction of hypoxia like conditions in nickel treated cells with subsequent selection for increased HIF-1-dependent transcription might be involved in nickel induced carcinogenesis (Salnikow and Costa, 2000; Salnikow et al 2000, Zhou et al 1998).

2.3.2. Chromium

Chromium is a naturally occurring element found in rocks, soil, plants, animals and in volcanic dust and gases. It occurs primarily in the trivalent state (III), which is the most stable reduced form, or in the hexavalent state (VI), which is a strong oxidizing agent. Elemental chromium (O) does not occur naturally on earth. (Barceloux, 1999).

Biologically chromium is an essential nutrient required for sugar and fat metabolism. Normal dietary intake of chromium for humans is suboptimal. The estimated safe and adequate daily dietary intake for chromium is 50-200 \(\mu\)g. However, most diets contain less than 60\% of the minimum suggested intake of 50\(\mu\)g. Insufficient dietary intake of chromium leads to signs and symptoms that are similar to that observed for diabetes and cardiovascular diseases. Supplemental chromium, given to people with impaired glucose tolerance or diabetes leads to improved blood glucose, insulin and lipid variables. Chromium has also been shown to improve lean body mass in humans. Response to chromium depends upon its form and amount of supplemental chromium. Trivalent chromium has a very large safety range and there has been no
documented sign of chromium toxicity in any of the nutritional studies at levels up to 1mg/day (Anderson, 1997).

2.3.2.1 Exposure to Chromium

Anke et al (1997) studied the dietary intake of chromium in healthy humans in different years (1988, 1992, 1996), seasons of the year (summer, winter), in man and woman, in lactating and non-lactating women, in different age groups, in different body weight groups, in consumers of mixed diets and vegetarians and in populations from Germany and Mexico. A total of 18 test populations were studied using seven day food records and duplicate methods of food sampling for analysis. The overall chromium intake was >50μg/day which satisfied the basal (20 μg/day) and recommended (33 μg/day) chromium uptake. Seasons and age had no effect on chromium intake. The chromium intake in men was 24% higher than in women due to higher dry food matter intake. Lactating women consumed more chromium than non-lactating women due to higher dry matter intake. Persons with higher body weights consume more chromium due to selection of foods with higher dry content. Chromium intake in vegetarians was higher than in mixed diet eaters (Anke et al 1997).

Chromium levels in wine ranged from 7.0 to 90.0 μg per litre in red wine, from 6.6 to 43.90 μg/l in white wine, from 7.3 to 14.7 μg/l in rose wine and from 10.5 to 36.0μg/l in champagne. In grapes, chromium levels ranged from 24.2 to 64.6μg/kg (Teisseder et al 1996).
Chromium metal is mostly used in production of special (stainless) steels and is also used to electroplate other metals. Chromium is present in high concentration in cement, where it may cause contact dermatitis. Chromium compounds are used as paint pigments and dyers, as a catalyst, to make magnetic tape, in tanning, in wood impregnation as a wood preservative and in safety match production (IPCS, 1987).

Occupational exposure of chromium to humans occurs mostly through extraction, production of ferrochrome (an iron-chromium alloy containing about 60% chromium, which is usually the main source of chromium, as the pure metal is not required for the production) pigment manufacture, electroplating, and welding (Bonde and Christensen, 1991). Generally chromium is found in non-industrial environments in air at 10ng/m³, in soil at concentrations of 10 to 90nm, and in fresh water at 1 to 10 µg/l. Under such conditions chromium is found as the chromate. Chromium is found in the air of industrial cities upto 70 ng/m³ and in water upto 25 µg/l concentrations.

Exposure to hexavalent chromium results in its uptake by the red blood cells with subsequent reduction in the trivalent species. Differential estimation of plasma and red cell levels of chromium could provide a sensitive internal indication of exposure to the hexavalent form. There is an indication that chromium levels are raised in hair after exposure (Winder et al 1997).

2.3.2.2 Chromium Carcinogenesis
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Chromate is one of the best documented human and animal carcinogens. Occupational exposure to chromium compounds has been widespread and many studies of the biological effects of chromium have been conducted (Stern 1982; Bianchi et al 1983; DeFloro and Wetterhahn, 1989). However, because of its complex intracellular metabolism, molecular mechanisms of chromium-induced genotoxicity are not thoroughly understood.

Chromium exists in a number of oxidation states, of which only Cr (VI) and Cr(III) are environmentally stable. While chromium (VI) as chromate, is biologically active, because it is taken up by cells and it rapidly reduces through relatively unstable Cr (V) and Cr (IV) intermediates to kinetically stable Cr(III) species (Arslan et al 1987). Because Cr (VI) is cellularly reduced, numerous studies have attempted to determine which intracellular form of chromium is the ultimate carcinogenic and/or mutagenic species (Lofroth, 1978; Macrae et al 1979; Whiting et al 1979). Cr(VI) compounds are biologically efficacious, while studies of Cr(III) compounds yield conflicting results. This is in part because chromium (III) species are not actively taken up by cells (Leonard and Lauwerys, 1980). However Cr(III) is the final stable intracellular valence state of carcinogenic and mutagenic Cr (VI) (DeFloro and Wetterhahn, 1989). In biological materials, as much as 90% of the cellular chromium is present as the trivalent species (Schreoder et al 1962).

Chromium(III), the most abundant form of chromium in the environment, does not readily cross cell membranes and is relatively inactive in vivo. Chromium(VI), the most biologically active species, is readily taken up by cells.
as the chromate anion through membrane anionic transporters and is reduced intracellularly via reactive intermediates to stable Cr (III) species. Cr(VI) does not react with biological macromolecules, however both Cr(III) and the reductional intermediate Cr(V) are capable of making co-ordinate covalent interactions with macromolecules. Cr(III) forms tight complexes with biological ligands such as DNA and proteins which are slow to exchange. In vitro CrCl$_3$.6H$_2$O primarily interacts with DNA to form outer shell charge complexes with the DNA phosphates. Reduction of chromate by a reducing agent such as glutathione, is a prerequisite for the generation of bonds between the metal and constituents of the cell nuclei in vitro. Chromium adducts with nuclei are probably one cause of DNA lesions and mutations. (Snow, 1991; Snow, 1994; Singh et al 1998) Hexavalent chromate is isostructural with phosphate and sulphate, it is readily taken up by the gastrointestinal tract and penetrates to many tissues and organs throughout the body. From the epidemiological studies there is suggestive evidence that hexavalent chromium causes increased risk of lymphomas, leukemia, hodgkins and cancers of bone, prostate, stomach, genital organs, kidney and urinary bladder, reflecting the ability of hexavalent chromate to penetrate all tissues in the body. A high accumulation of Cr(III) in all tissues and organs is a strong indication of the wide toxic potential of exposure to soluble hexavalent Cr in the drinking water and in the ambient environment (Costa, 1997).

Chromate is mutagenic in a number of bacterial systems (Nishioka, 1975; Kanematsu et al 1980). When tested in the Ames histidine reversion assay using
salmonella typhimurium, Cr(VI) is most potent in producing base pair substitutions in the A.T. specific strains TA 104 and TA 102 (DeFloro and Wetterhahn, 1989). These strains are especially susceptible to oxidative damage and it is very likely that a major component of chromate genotoxicity is mediated by active oxygen species produced during intracellular chromate reduction. Both mutagenicity and carcinogenicity of Cr(VI) are also well established in mammalian cell cultures and in vivo (Bianchi et al 1983, Sen and Costa, 1986; Sen et al 1987; Sugiyama et al 1986). In animal experiments, the administration of Cr(VI) compounds results in tumor formation at the site of injection or implantation (Bianchi et al 1983).

Chronic inhalation experiments of chromium aerosol were reported to cause adverse effects in the lung. Glaser and co-workers (1985) found that, although exposure to low chromium level caused activation of alveolar macrophages, high-level exposure inactivated it. They also showed that Cr$_5$O$_{12}$ aerosol was much more toxic to lung and blood cells than Na$_2$Cr$_2$O$_7$ aerosol (Glaser et al 1986). Hepatic and renal damages have been reported in the acute phase of chromium parenteral injection at dose levels of 7.9 to 15.8 mg chromium per kg in rats (Tsapakos et al 1981; Standeven and Wetterhahn, 1991; Mikalsen et al 1991; Kim and Na, 1991).

In the subacute to chronic phase, reproductive tissues were also affected. Serial i.p. injection of 1 to 4 mg chromium per kg as dichromate for 5 to 90 days caused pathological changes in testis cells or altered enzyme activities in rats (Ernst, 1990; Saxena et al 1990; Murthy et al 1991). Vyskocil et al (1993) showed
that female rats manifested renal dysfunction after 6-month exposure to 25-ppm Cr (as chromate) in drinking water, but males did not. When pregnant mice were exposed to K₂Cr₂O₇ in drinking water containing 250 to 1000 ppm chromium throughout the gestation period, embryonic death and malformation of offsprings occurred (Trivedi et al 1989). The effects of long-term ingestion of chromium chloride (trivalent compound) and potassium dichromate (hexavalent compound) was investigated by Bataineh et al (1997) on sexual behaviour, aggressive behaviour and fertility in male rats. Adult male rats were exposed to chromium chloride and potassium dichromate in drinking water at a concentration of 1000 ppm for 12 weeks. The exposure of male rats to chromium chloride and potassium dichromate reduced the number of mounts. The exposure of male rats to potassium dichromate increased the time of ejaculation. On the other hand, the exposure of male rats to chromium chloride and potassium dichromate increased the post ejaculatory interval. In conclusion, the long-term ingestion of chromium chloride and potassium dichromate would have adverse effects on sexual behaviour in adult male rats.

At the genomic level, chromium genotoxicity manifests as gene mutations, several types of DNA lesions and inhibition of macromolecular synthesis. At the cellular level, chromium exposure may lead to cell cycle arrest, apoptosis, premature terminal growth arrest or neoplastic transformation. Chromium induces DNA-DNA interstrand crosslinks. The tumor suppression gene, p53, and oxidative processes are some of the major factors that may play a significant role
in determining the cellular outcome in response to chromium exposure (Singh et al. 1998).

2.3.2.2.2 Effect of chromium on DNA polymerase activity

Snow and Xu (1991) investigated the effects of chromium(III) binding on DNA replication and polymerase processivity in vitro. Chromium ions bind in a dose-dependent manner to DNA. Micromolar concentrations of free chromium inhibit DNA replication, but if the unbound chromium is removed by gel filtration, the rate of DNA replication by polymerase I (klenow fragment) on the chromium-bound template is increased greater than 6-fold relative to the control. This increase is paralleled by as much as a 4-fold increase in processivity and a 2-fold decrease in replication fidelity. These effects are optimum when very low concentrations of chromium ions are bound to the DNA (3-4 Cr(III) ions per 1000 nucleotide phosphates). Increased concentration of chromium leads to the production of DNA-DNA cross links and inhibition of polymerase activity. These results suggest that low levels of DNA bound chromium(III) ions may contribute to chromium mutagenesis and carcinogenesis by altering the kinetics and fidelity of DNA replication.

Chromium (VI) is taken up by the cells as the chromate anion and is reduced intracellularly via reactive intermediates to stable Cr(III) species (Snow, 1994). Chromium(III) forms tight complexes with biological ligands such as DNA and proteins, which are slow to exchange. In vitro CrCl₃ primarily interacts with DNA to form outer shell charge complexes with DNA phosphates. However, at micromolar concentrations, Cr(III) binds to a low number of saturable tight
binding sites on single-stranded M-13 DNA. Chromium interacts in a non-specific manner with the DNA and can form intermolecular DNA crosslinks. Although high concentration of Cr(III) inhibit DNA replication, micromolar concentration of Cr(III) can substitute for Mg$^{2+}$, weakly activate the klenow fragment of *E. coli* DNA polymerase I and acts as an enhancer of nucleotide incorporation. Alterations in enzyme kinetics induced by Cr(III) increase DNA polymerase processivity and the rate of polymerase bypass of DNA lesions. This results in an increased rate of spontaneous mutagenesis during DNA replication both *in vitro* and *in vivo*. These results indicate that chromium(III) may contribute to chromate-induced mutagenesis and may be a factor in the initiation of chromium carcinogenesis (Snow, 1994).

It was shown that trivalent chromium and hexavalent chromium in the presence of one of its primary in vivo reductants, ascorbate, can bind to DNA and form interstrand crosslinks capable of obstructing replication (Bridgewater *et al* 1998).

### 2.3.2.2.3. Chromium induced DNA damage

Chromium(VI) compounds induce chromosomal aberrations and increase the incidence of sister chromatid exchanges, as well as DNA-protein cross links (DPCs) and DNA strand breaks in cultured mammalian cells (Sen and Costa, 1986; Sen *et al* 1987; Sugiyama *et al* 1986).

Chromate compounds can cause damage in DNA or protein by generating DPCs. DPCs represent a "biomarker of chromate exposure" and early carcinogenic effects which quantitatively shows exposure to these agents.
Tsapakos et al (1981) detected DPCs in liver and kidney of chromium-injected rats, suggesting a relationship to the carcinogenicity and toxicity of Cr(VI). Increased DNA-protein cross-links were detected in lymphocytes and livers of rats given drinking water contaminated by 100 to 200 ppm Cr as chromate (Coogan et al 1991). In the study conducted by Lei et al (1995) DNA-protein cross links were demonstrated in WBCs, liver and kidney of rats which were actually treated with $K_2Cr_2O_4$. There was a close-dependent relationship between chromate exposure and DPCs in WBCs and liver.

Studies of DPCs in peripheral blood lymphocytes have been conducted with individuals who had higher exposure to chromate, including welders and with individuals who had lower levels of exposure such as residents living in a Cr-contaminated area in Jersey City, New Jersey (Costa et al 1996). Studies were also conducted in two Bulgarian cities (Jambol and Burgas) with different levels of air pollution and Cr(VI) exposure and in chrome platers in Bulgaria who had high exposure to chromate. DPCs in US welders and in individuals living in Hudson country, New Jersey around Cr-contaminated areas were significantly higher compared to matched controls. Chromium levels in RBCs of controls living in Burgas were in the order of 1 to 2 ppb chromium. However the chromium levels in Jambol ranged from about 2 to 7 ppb in RBCs of city residents to about 22 ppb in chrome platers. DPCs were saturated at about 7 to 8 ppb chromium in RBCs and cross-links correlated well only with chromium levels in RBCs (Costa et al 1996).
Dudek and Wetterhahn (1994) have shown that Chromium(VI) induced cytotoxicity and DNA damage occur via two distinct pathways: an oxidative pathway and direct chromium-DNA interaction. Treatment of rodent FAO cells, a hepatoma cell line possessing both high levels of catalase activity and endogenous glutathione, with increasing concentration of chromium(VI) and \( \text{H}_2\text{O}_2 \) produced dose dependent cytotoxicity as determined by incorporation of \(^3\text{H}\)-thymidine into DNA and by overall cell survival. When cells were pretreated with L-buthionine-[S,R]- sulfoximine in order to deplete intracellular glutathione, these were protected against Cr [VI] cytotoxicity but sensitised to \( \text{H}_2\text{O}_2 \) treatment. Cells that were pretreated with 3-amino-1,2,4-triazole to inactivate catalase activity, were significantly sensitised to both Cr(VI) and \( \text{H}_2\text{O}_2 \). When oxidative DNA damage was examined, single-stranded DNA breaks were increased in catalase depleted cells treated with Cr(VI), suggesting that reactive oxygen species may be responsible for the observed increase in cytotoxicity. These results suggest that two potential pathways exists for Cr(VI)-mediated genotoxicity. One pathway occurs through the involvement of glutathione, possibly by the formation of glutathione-Cr-DNA complexes, while the other pathway occurs via reactive oxygen intermediates with formation of DNA strand breaks (Dudek and Wetterhahn 1994). It was reported that Cr(IV)-GSH complex was also able to generate hydroxyl (\( \text{OH}^+ \)) radical in the presence of molecular oxygen in aqueous medium. Catalase inhibited the \( \text{OH}^+ \) radical generation while \( \text{H}_2\text{O}_2 \) enhanced it, indicating that the \( \text{OH}^+ \) radical was generated via a Fenton-
like reaction, H$_2$O$_2$ being generated as an intermediate in the reduction of molecular oxygen (Liu et al 1997).

Intermediates produced in the reaction of Chromium(VI) with dehydroascorbate cause single strand breaks in plasmid DNA (Stearns and Wetterhahn, 1997). Results suggest that it is not a single type of species that universally produces the DNA strand breaks observed in different Cr(VI) systems and that the reactivity of intermediates will depend on the chosen experimental conditions. Understanding this variability in Cr(VI) reactions may help to resolve the conflicting results from in vitro studies that are aimed at deciphering mechanisms of Cr(VI)-induced cancers.

DNA damage and sister chromatid exchange (SCE) frequencies were measured in lymphocytes of 39 welders and 39 controls exposed to chromium and nickel (Werfel et al 1998). The welders showed a significantly higher rate of DNA single-strand breakages and significantly elevated SCE values. DNA single-strand breakage and DNA protein cross-links show different increases depending upon the exposure levels from chromium and nickel.

Borges and Wetterhahn (1991) have shown that chromium bound to DNA alters cleavage by restriction endonucleases (RE). RE cleavage of PBR322 DNA was studied after in vitro formation of chromium-DNA adducts by treatment of the DNA with potassium dichromate in the presence of the thiols, glutathione, dithiothreitol or β-mercaptoethanol. The abilities of certain restriction endonucleases to cleave DNA was affected markedly by the presence of chromium DNA adducts. Chromium-DNA adduct formation affected cleavage at
the Ndel endonuclease sites in PBR322 and SV40 DNA and at the unique BamHI endonuclease site in SV40 DNA, even when the chromium-DNA adducts did not induce detectable DNA structural distortion. Inhibition of Ndel endonuclease cleavage of PBR322 and enhancement of BamHI cleavage of SV40 occurred with glutathione-chromium-DNA complexes generated by reaction of DNA with chromium(VI) and glutathione. In contrast enhancement of Ndel endonuclease cleavage of PBR322 and SV40 and inhibition of BamHI cleavage of SV 40 occurred with chromium-DNA complexes generated by reaction of DNA with chromium (VI) and β-mercaptoethanol or dithiothreitol. These results suggest that chromium bound at or near specific enzyme recognition sites interfere with normal enzyme activity. Low levels of bound chromium (generally <5x10^-3 chromium/nucleotide) which did not alter DNA conformation as detected by agarose gel electrophoresis, did not interfere with the endonuclease cleavage of DNA by BamHI, NarI, Ndel or Dral. At high chromium-DNA binding levels (>0.2 chromium/nucleotide) which induced major DNA structural distortion, cleavage by all REs tested was inhibited. Since a preference for cleavage inhibition at a particular DNA recognition sequence was not observed, it is possible that endonuclease inhibition resulted from overall DNA conformational distortions induced by chromium binding. Thus chromium-DNA adducts can modulate the interaction of enzymes with DNA. Disruption of normal DNA-protein interactions by chromium-DNA adducts may affect important cellular processes such as replication and transcription.
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Kaltreider et al. (1999) have reported that arsenic and chromium significantly alter nuclear binding levels of transcription factors, AP-1, NF-kappa B, Sp1 and YB-1 to their respective cis-acting elements. Cr(VI)-mediated activation of transcription factors may be involved in the mechanism of Cr(VI) induced carcinogenicity (Kim and Yurkow, 1996; Kaltreider et al. 1999).