Review Article

Nickel, its adverse health effects & oxidative stress

K.K. Das, S.N. Das* & S.A. Dhundasi

Environmental Health Research Unit, Department of Physiology, Al Ameen Medical College &
*B.L.D.E.A's Dr P.G. Halakatti College of Engineering & Technology, Bijapur, Karnataka, India

Nickel-induced toxicity and carcinogenicity, with an emphasis on the generation and role of reactive oxygen species is reviewed. Nickel is a known haematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic , hepatotoxic and carcinogenic agent. This article presents a selective review on nickel and effect of its acute, subchronic and chronic doses on certain metabolically active tissues in human as well as animals. Nickel exposure causes formation of free radicals in various tissues in both human and animals which lead to various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulphhydryl homeostasis. The primary route for nickel toxicity is depletion of glutathione and bonding to sulphhydryl groups of proteins. Nickel homeostasis, nickel-induced activation of signaling pathways and the protective role of enzymatic and non-enzymatic antioxidants against nickel toxicity and carcinogenicity are also discussed.

Introduction

Nickel is a silvery white metal that takes on a high polish. It is a transition metal, hard and ductile. It occurs most usually in combination with sulphur and iron in pentlandite, with sulphur in millerite, with arsenic in the mineral nickeline, and with arsenic and sulphur in nickel glance[1]. Nickel is one of the five ferromagnetic elements. Nickel is also a naturally magnetostrictive material, meaning that in the presence of a magnetic field, the material undergoes a small change in length[2]. The properties of nickel and its environmental distribution have been summarized by the US Agency for Toxic Substances and Disease Registry (ATSDR)[3]. Nickel is used in a wide variety of metallurgical processes such as electroplating and alloy production as well as in nickel-cadmium batteries. There is evidence suggesting that nickel may be an essential trace element for mammals[4]. Nickel is primarily found combined with oxygen or sulphur as oxides or sulphides that occur naturally in the earth's crust. Nickel combined with other elements is present in all soils, in meteorites, and is emitted from volcanoes. As for most metals, the toxicity of nickel is dependent on the route of exposure and the solubility of the nickel compound[5]. The purpose of this review is to provide a detailed overview of current state of knowledge of the nickel toxicity and in the formation of reactive oxygen and nitrogen species and tissue damage and role of some antioxidant supplementation.

Metabolism and disposition

Absorption: Nickel is believed to play a role in physiological processes as a co-factor in the absorption of iron from the intestine. The interaction between nickel and iron occurs only under certain conditions. Nickel increased the absorption of iron from the diet in iron deficient rats (female), but only when dietary iron was in the unavailable ferric form, whereas a mixture of ferrous and ferric sulphates (60% ferric to 40% ferrous) as a supplement to the diet did not elicit any effect[6]. In short- and long-term studies of animal administered various soluble nickel salts orally, nickel was found primarily in the kidneys. The relative tissue concentrations were kidneys > lungs> liver >heart> testes[7,8]. Oral administration of Ni^{+2} found to accumulate higher in the spinal cord than in the cerebellum or frontal cortex[9]. Pulmonary absorption is the major route of concern for nickel-induced toxicity. Nickel may be absorbed as the soluble nickel ion (Ni^{+2}) while sparingly soluble nickel compounds may be phagocytized. The chemical form and its deposition site (determined by size, shape, density, and electrical charge of the nickel particles) in the lungs will affect the extent of absorption[3]. Nickel may be removed from portions of the respiratory tract via mucociliary transport resulting in the material entering the gastrointestinal tract. Although nickel is poorly absorbed from the gastrointestinal tract but dietary exposure and exposure via drinking water provide most of the intake of nickel and nickel compounds[4-5]. As reviewed by the Environmental Protection Agency (EPA) (1986), humans and animals absorb approximately 1-10 per cent of dietary nickel[10]. Similar values were reported for drinking water exposure and gavage administration[3]. Nickel metal is poorly absorbed dermally but some nickel compounds such as nickel chloride or nickel sulphate can penetrate occluded skin resulting in up to 77 per cent absorption within 24 h[3]. Pharmacokinetic studies in humans indicate that nickel is absorbed through the lungs[11-13], gastrointestinal tract[14-16], and skin[17,18]. Following absorption from the lungs and the gastrointestinal tract, nickel is excreted in the urine[19-21]. A 70 kg reference man contains 10 mg of nickel, giving an average body concentration of 0.1 ppm. Reference values for nickel in healthy adults is 0.2 µg/l in serum and 1-3 µg/l in urine[3]. A National Health and Nutritional Examination Survey II of hair found mean nickel levels of 0.39 ppm, with 10 per cent of the population having levels >1.50 ppm[3]. About 20-35 per cent of the inhaled nickel that is retained in the lungs is absorbed into the blood. Absorption of nickel following oral exposure has been shown to vary (3-40%) depending on whether the nickel was in drinking water or food. It was noticed that a greater absorption of nickel occurred in drinking water. Fasting individuals have also been shown to absorb more nickel from the gastrointestinal tract. Most of the absorbed nickel is excreted in the urine, regardless of the route of exposure[14].

Distribution: Increased levels of nickel were found in the lungs, nasal septum, liver, and kidneys of workers inhaling nickel[22-24]. Animal data indicate that after inhalation, nickel particles can remain in the lungs (nickel oxide) or be absorbed and then excreted in the urine (nickel sulphate). High levels of nickel were also found in the liver, heart, lungs, fat, peripheral nervous tissue, and brain[7-9,25]. Nickel is known to bind to specific proteins and/or amino acids in the blood serum and the placenta. These ligands are instrumental in the transport and distribution of nickel in the body.

Metabolism: Nickel is not destroyed in the body, but its chemical form may be altered. The metabolism of nickel is most appropriately viewed in light of its binding to form ligands and its transport throughout the body. Much of the toxicity of nickel may be associated with its interference with the physiological processes of manganese, zinc, calcium, and magnesium[5]. Various disease states (myocardial infarction and acute stroke) and injuries (burn injury) are associated with altered transport and serum concentrations of nickel[3]. Nickel concentrations in surface water and groundwater range between 3 and 10 µg/l. Nickel levels in drinking water in the United States generally range from 0.55 to 25 µg/l and average between 2 and 4.3 µg/l[3]. Based on these average nickel concentrations and a reference water intake of 2 l/day, the estimated average intake of nickel from drinking water ranges from 4 to 8.6 µg/day. Ambient air concentrations of nickel range between 7 and 12 ng/m^3, mainly in the form of aerosols and can be as high as 150 ng/m^3 near point sources. Based on 1996 air quality data, EPA has reported average U.S. ambient air levels of 2.2 ng/m^3. Ambient air levels of nickel are expected to be higher in urban air than in rural air. Concentrations of nickel in indoor air are generally 10 ng/m^3 [26].

Excretion: In humans most ingested nickel is not absorbed and is eliminated predominantly in the faeces. Some of the nickel absorbed from the gastrointestinal tract is excreted in the urine and is associated primarily with low molecular weight complexes that contain amino acids. In human nickel can also be eliminated through sweat and milk[27]. It has been reported that

faecal nickel is usually about 100 times that of urinary nickel and represents the removal of unabsorbed nickel[3,4]. Urinary nickel levels of 0.4-6.0 g/l in non-exposed, healthy adults were reported by Sunderman *et al*[27,28] while levels as high as 400 g/l were also reported by Sunderman *et al*[29] for workers occupationally exposed to nickel and nickel compounds.

Nickel toxicities and health effects: An overview

The adverse health effects of nickel depend on the route of exposure (inhalation, oral, or dermal) and can be classified according to systemic, immunologic, neurologic, reproductive, developmental, or carcinogenic effects following acute (01day), subchronic (10-100 days), and chronic (100 days or more) exposure periods. The most common harmful health effect of nickel in humans is an allergic skin reaction in those who are sensitive to nickel. Nickel is the most observed cause of immediate and delayed hypersensitivity noticed in occupationally exposed as well in the general population. The metal is not only an allergen but also a potential immunomodulatory and immunotoxic agent in humans[30]. Based on studies of nickel workers and laboratory animals, all nickel compounds, except for metallic nickel, have been classified as human carcinogens by the International Agency for Research on Cancer (IARC)[31] and the U.S. Department of Health and Human Services[32].

Health risk assessment on nickel: There are five priority substances which are selected by WHO for the nickel risk assessment. They are nickel powder, nickel sulphate, nickel chloride, nickel carbonate and nickel nitrate. Nickel powder (T; R48-23) has been classified in chronic toxicity classification as per environmental risk assessment report on nickel. $NiSO_4$, $NiCl_2$, $NiCO_3$ and $NiNO_3$ are classified as carcinogen class I (by inhalation), reproductive toxicants class II (may cause harm to unborn children) and chronic toxicants (T; R48-23). If particle size of nickel powder found to be less that 0.1 mm, it is classified as T; R52-53 (harmful to the aquatic environment)[33].

Acute toxicity (01 day): The accidental inhalation of nickel carbonyl generally causes acute toxic effects in two stages, immediate and delayed. The immediate symptoms include headache, vertigo, nausea, vomiting, insomnia, irritability, which usually last a few hours, followed by an asymptomatic interval of 12 h to 5 days. Then delayed symptoms appear-tightness of the chest, nonproductive cough, dyspnoea, cyanosis, tachycardia, palpitations, sweating, visual disturbances, vertigo,

weakness, and lassitude[34]. A fatal case of nickel poisoning was reported for a 2 ½ yr old girl who had ingested 15 g of nickel sulphate[35]. The cause of death was cardiac arrest. Death due to nickel-induced adult respiratory distress syndrome (ARDS) was reported for a worker spraying nickel using a thermal arc process[36]. Nausea, vomiting, abdominal pain, diarrhoea, headache, cough, shortness of breath, and giddiness were reported for workers of an electroplating plant who drank water contaminated with nickel chloride and nickel sulphate (1.63 g/l)[37]. Signs and symptoms of toxicity lasted for up to 2 days with uneventful recoveries for all 32 workers. In male rats, a single dose of nickel chloride injection caused a profound and consistent increase in circulating prolactin levels after one day and lasted for four days[38]. Kidney injury and frank haematuria were also observed in acute nickel toxicity[39]. Water-soluble nickel compounds have been shown to be more acutely toxic than the less soluble ones. The single dose oral LD_{50} in rats for the less soluble nickel oxide and subsulphide were > 3,600 mg Ni/kg b wt, whereas the oral LD_{50} for the more soluble nickel sulphate and nickel acetate ranged from 39 to 141 mg Ni/kg b wt in rats and mice[40].

Subchronic toxicity (10-100 day): In an evaluation of workers welding high-nickel alloys, it was reported that 6-wk exposure to nickel fumes (0.07 to 1.1 mg nickel/m³) caused an increase in airway and eye irritations, headaches, and tiredness[41]. Weber and Reid[42] reported significant reductions in body weight gain, decreased body weight, and signs of hepato and renal toxicities in animals as a result of daily exposure to nickel via diet or gavage. Weischer *et al*[43] reported that oral administration of nickel as $NiCl_2$ in male rats over a period of 28 days at concentration of 2.5, 5.0 and 10.0 µg /ml in drinking water (0.38, 0.75, or 1.5 mg/kg/day) resulted in significant dose-dependent hyperglycaemia, decrease in serum urea and significant increase in urine urea. At 0.75 mg/kg doses increased leukocyte count was also observed[43]. Whanger[25] reported reduced blood hamoglobin and PCV after nickel exposure. Toxic symptoms like lethargy, ataxia, hypothermia, salivation, diarrhoea were observed in the nickel treated rats at 10 mg/kg/d doses[30]. The mortality rate among rats are very high in dose-dependent nickel treatment[44]. Obona *et al*[45] reported that in adult male Sprague-Dawley rats given $NiSO_4$ at 0, 0.02, 0.05 and 0.1 per cent or 0, 44.7, 111.75 and 223.5 mg/l respectively (estimated doses of 0, 5, 12.5 and 25 mg/kg/day) in their drinking water for 13 wk, both the

absolute and relative liver weights in the 12.5 mg/kg and 25 mg/kg groups were significantly decreased. Total plasma protein, plasma albumin and globulins and plasma glutamic pyruvic transaminase activity were found significantly decreased in the highest dose group[45]. It was noticed that exposure of dietary nickel sulphate hexahydrate (100, 1000, or 2500 ppm) to dogs for 2 years failed to produce significant signs of compound-related toxicity[7]. A 91-day study using rats given nickel chloride hexahydrate by gavage at doses of 5, 35, or 100 mg nickel/kg/day resulted in the death of all rats in the high-dose group by day 78. An increase in white blood cell counts for those in the 35 mg/kg/day group at an interim sacrifice, and an elevated platelet count and decreased blood glucose level in the 35 mg/kg group at final sacrifice were also observed[46]. Renal tubular degeneration was reported for rats receiving dietary nickel acetate (0.1 to 1.0%) for several weeks. Twelve women who were occupationally exposed to soluble nickel compounds (0.75 mg Ni/m^3 average concentration) showed increased urinary levels of total protein, β_2-microglobulin, retinal binding protein, and N-acetyl-β-D-glucosaminidase (NAG)[47]. Although altered biomarkers reflected tubular dysfunction, no effect was found on markers of glomerular function, urinary albumin levels, or transferrin levels.

A study of 17 electroforming workers suffering from occupational dermatitis following heavy exposure to nickel revealed no evidence of proteinuria[48]. A 13-wk inhalation study conducted by the Inhalation Toxicology Research Institute compared the effects of exposure of rats and mice to nickel sulphate hexahydrate, nickel subsulphide, and nickel oxide at occupationally relevant exposure concentrations[49]. Severity of inflammation and fibrosis of the lungs and alveolar macrophage hyperplasia corresponded to the water solubility of the nickel compounds with nickel sulphate being the most toxic, followed by nickel subsulphide and nickel oxide[49]. Haley *et al*[50] reported immunotoxic effects in mice following 13-wk exposures to various nickel compounds. Nickel subsulphide (1.8 mg nickel/m^3) decreased activity levels of natural killer cells while both nickel oxide (0.47, 2.0, and 7.9 mg nickel/m^3) and nickel subsulphate (0.45 and 1.8 mg nickel/m^3) inhibited phagocytic ability of alveolar macrophages[50]. In studies with mice and rats, Smialowicz *et al*[51-53] found that nickel chloride affected T cell-mediated immune responses (but not humoral immune responses) and that natural killer cells were target cells of the immunotoxic effects of nickel. Alterations in innate (or non specific) and acquired immunity have been observed in animals. Several studies examined alveolar macrophage functions. A significant reduction in macrophage phagocytic activity was observed in rats exposed to an unspecified concentration of nickel chloride for 2 h[54] or in mice exposed to 0.47 mg Ni/m^3 as nickel oxide or 0.45 mg Ni/m^3 as nickel subsulphide 6 h/day, 5 days/wk for 65 days[55]. No alteration of macrophage phagocytic activity was observed in mice exposed to <0.45 mg Ni/m^3 as nickel sulphate 6 h/day, 5 days/wk for 65 days[55].

Chronic toxicity (>100 day): Most chronic inhalation exposures involve occupational exposure to nickel dust or nickel vapors resulting from welding nickel alloys. Generally, chronic inhalation exposure to nickel dusts and aerosols contribute to respiratory disorders such as asthma, bronchitis, rhinitis, sinusitis, and pneumoconiosis[56]. Based on analyses of studies completed prior to 1975, the National Academy of Sciences (NAS) concluded that nickel refinery workers demonstrated an increase in the incidence of pulmonary and nasal cavity cancers, specifically epidermoid, anaplastic, and pleomorphic cancers[57]. Enterline and Marsh[58] showed that there was an excess risk of nasal sinus cancer in nickel refinery workers. A lifetime exposure of rats to nickel oxide (42 mg nickel/m^3) produced emphysema and other proliferative and inflammatory changes[59]. It was reported by Vyskocil *et al*[47] that drinking water contains $NiSO_4$ at 100 mg/l exposure to both male and female rats causes significant increases in kidney weights. Urinary excretion of albumin increased significantly in female rats but the increase was marginalized in male rats. Male and female Wistar rats fed diets containing nickel as $NiSO_4$ at 0, 100, 1000, 2500 ppm (0, 5, 50 and 125 mg/kg/day) for 2 yr showed significant body weight reduction in both male and female rats[47]. Schroeder *et al*[60] observed focal myocardial fibrosis in rats exposed to nickel at 5 ppm for 18 months. Ambrose *et al*[7] reported increased heart weights without any reported histological changes of the heart tissue in rats exposed to 75 mg/kg/day as $NiSO_4$ in a 2 year study. No clinical evidence of developmental or reproductive toxicity was reported for women working in a nickel refinery[61]. Chashschin *et al*[62] reported on possible reproductive and developmental effects in humans of occupational exposure to nickel (0.13-0.2 mg nickel/m^3). The NTP[63-65] studies allow for the comparison of the toxicity of nickel sulphate, nickel subsulphide, and nickel oxide in rats and mice. Following acute- or intermediate-duration exposure, the toxicity of the different nickel

compounds is related to its solubility, with soluble nickel sulphate being the most toxic and insoluble nickel oxide being the least toxic. The difference in the toxicity across compounds is probably due to the ability of water-soluble nickel compounds to cross the cell membrane and interact with cytoplasmic proteins. In contrast, the severity of inflammatory and proliferative lesions following chronic exposure was greater in rats exposed to nickel subsulphide or nickel oxide, as compared to nickel sulphate. Additionally, parenchymal damage secondary to inflammation was evident in the rats exposed to nickel subsulphide and nickel oxide, but not nickel sulphate[63-65].

Some specific aspects of nickel toxicities

Genotoxicity: Biggart and Costal[74] have observed that nickel is a nongenotoxic agent. In contrast, both *in vivo* and *in vitro* tests on higher organism cell it was observed that nickel produces some genetic abnormalities like DNA strand break, DNA-protein cross links, nucleotide excision, single gene mutations, sister chromatid exchanges, micronuclei, nucleic acid concentration alteration and cell transformation[5,66,67]. Investigators conducting epidemiology studies have reported a higher incidence of chromosomal aberrations in nickel workers compared to controls[20,68]. Both *in vitro* and *in vivo* studies in mammals indicate that nickel is genotoxic[69-72]. The available evidence suggests that nickel does not alter the frequency of gene mutations in non mammalian organisms[73-75], although some studies have found gene mutations[76]. Mixed results for gene mutations have been found in mammalian test systems. Increases in the frequency of gene mutations have been found at the HGPRT locus in Chinese hamster V79 cells[77] but not in Chinese hamster ovary cells[78]. An increase in gene mutation frequency has also been found in Chinese hamster ovary AS52 cells (*grp* locus)[79], mouse lymphoma cells[80,81] and virus-infected mouse sarcoma cells[82-83]. Gene mutation frequency was not affected in transgenic mouse and rat respiratory tissue following inhalation exposure to nickel subsulphide[84]. Nickel enhances the genotoxicity of ultraviolet light, X-rays, *cis-* and *trans-*platinum, and mitomycin C. *In vitro* studies in HeLa cells suggest that nickel inhibits the incision step in excision repair[85] while studies using Chinese hamster ovary cells suggest that nickel inhibits the ligation step of excision repair[86]. Protein seems to be the primary target for nickel insult[87]. The threshold concentration of nickel induced mutagenesis is impossible to establish at present because in most studies the lowest concentrations used were near those that cause high cytotoxicity. Nickel causes increased level of endogenous cellular hydrogen peroxide and its short lived reactive oxygen species[87,88]. Nuclear protein damage caused by nickel reduces the enzyme activity needed for DNA replication, transcription, recombination and repair.

Developmental toxicity: There are limited data on the potential developmental toxicity of nickel in humans. An increase in structural malformations was observed in infants of women who worked in a nickel hydrometallurgy refining plant[89]. Decreased foetal body weight was observed in offspring of rats exposed to high levels of nickel via inhalation during gestation[90]. Developmental effects have also been observed in animals following parental administration of nickel[91]. The available animal data on developmental toxicity provide suggestive evidence that the developing foetus and neonates are sensitive targets of nickel toxicity. Male-only exposure to 3.6 mg Ni/kg/day as nickel chloride in drinking water for 28 days resulted in decrease the number of pups born alive (2.7/dam versus 10.2/dam in controls), the number of pups surviving until postnatal day 4 (56% versus 100% in controls), and litter size at postnatal day 21 (1.3 pups versus 9.2 pups in controls)[92]. An increase in spontaneous abortions was observed in female mice exposed to 160 mg Ni/kg/day as nickel chloride in drinking water on gestational days 2-17[93]. Decreases in pup body weights were reported in the offspring of rats exposed to 90 mg Ni/kg/day, 30, and 55 mg Ni/kg/day[7,94-95]. Neither Ambrose *et al*[7] nor the Research Triangle Institute (RTI)[94,95] multi generation studies found any significant, nickel-related gross abnormalities in the surviving offspring of rats exposed to nickel.

Haematotoxicity: No human studies were located regarding haematological effects after inhalation or dermal exposure to nickel. Regarding oral exposure, one study reported a transient increase in blood reticulocytes in workers who drank water from a water fountain contaminated with nickel sulphate, nickel chloride, and boric acid (estimated dose of 7.1-35.7 mg Ni/kg)[37]. A number of haematological alterations were observed in studies by Weischer *et al*[43] and National Toxicology Programme (NTP)[63-65]. A transient increase in blood reticulocytes was observed in workers who were hospitalized after drinking water during one work shift from a water fountain contaminated with nickel sulphate, nickel chloride, and boric acid[37]. Intermediate duration exposure to >0.7 mg Ni/kg/day as various nickel salts produced haematological effects in rats. Effects included a decrease in haemoglobin level in rats exposed to 25 mg Ni/kg/day as nickel acetate in the

diet for 6 wk[25]. An increase in leukocyte levels was found in rats exposed to 0.49 mg Ni/kg/day as nickel chloride in drinking water for 28 days[43] and also an increase in platelet counts in rats administered via gavage 8.6 mg Ni/kg/day as nickel chloride for 91 days was observed[46]. No haematological effects were observed in rats treated with nickel sulphate in the diet at a dose of 187.5 mg Ni/kg/day for 2 yr[7]. Low haematocrit levels were also observed in dogs after chronic dietary exposure to 62.5 mg Ni/kg/day as nickel sulphate[7]. A decrease in hematocrit level was observed in male rats continuously exposed to 0.178 or 0.385 mg Ni/m^3 as nickel oxide for 28 days. Another study using nickel sulphate in rats by Das *et al*[96] found that intraperitoneal administration of the compound significantly decreased the erythrocyte count, haematocrit value (PCV%), and haemoglobin concentration when compared with untreated controls. The authors also reported a significant increase in clotting time, followed by decrease in platelet and leukocyte count after nickel treatment in rats. Such a decrease could result in nickel-induced anaemia (non-regenerative anaemia) arising from injury of haematopoietic stem cells. Nickel sulphate probably decreases all types of blood cells in rats by inhibiting bone marrow activity[96].

Immunotoxicity: Nickel is capable of evoking dual responses in the human immune system, sometimes in the same subject[97]. Experiments conducted in humans and in rodents have shown that nickel exhibits both immunomodulatory and immunotoxic effects. Allergic dermatitis and immunologic urticaria can be seen in the area of contact as well as at distant sites. A systemic allergic reaction to nickel can manifest as both immune and allergic reactions[98-100]. Although a metal ion like nickel is too small to be antigenic by itself, the metal can oxidize to a low molecular weight substance called a *hapten,* which is non-immunogenic alone but can elicit an immune response when joined with a larger molecule, like tissue protein. The binding of the metal modifies the native protein configuration, and the hapten-specific T cells in the host immune system recognize the altered protein as a non-self antigen[30]. Number of immunological and lymphoreticular effects have been reported in humans and animals exposed to nickel. In 38 production workers exposed to nickel (compound not specified), significant increases in levels of immunoglobulin G (IgG), IgA, and IgM and a significant decrease in IgE levels were observed[101-102]. Significant increases in other serum proteins, which may be involved in cell-mediated immunity (including α1-antitrypsin, α2-macroglobulin, ceruloplasmin) were also noticed. Nickel and chromium significantly depressed the circulating antibody response of rats immunized with a viral antigen, with the greatest decrease in antibody titres noted in animals receiving the metal two weeks before the initial antigen dose[103,104]. Several studies have examined the relationship between nickel exposures and acquired immune function. An increase in susceptibility to Streptococcal infection was observed in mice exposed to 0.499 mg Ni/m^3 as nickel chloride or 0.455 mg Ni/m^3 as nickel sulphate for 2 h[54]. Mice exposed to 0.657 mg Ni/m^3 as nickel chloride also developed septicaemia from the Streptococci infection and had a reduced ability to clear the inhaled bacteria[54].

Nickel and chromium significantly depressed the circulating antibody response of rats immunized with a viral antigen, with the greatest decrease in antibody titers noted in animals receiving the metal two weeks before the initial antigen dose[103,104]. In a mouse model, both IgG and IgM antibody responses to Keyhole Limpet Hemocyanin (KLH) were significantly depressed *in vivo* in nickel fed animals[105].

Neurotoxicity: Neurological effects (giddiness, weariness) were reported in individuals accidentally exposed to nickel and boric acid in drinking water[37]. Neurological signs (lethargy, ataxia, prostration) were observed in dying rats treated with nickel for 3 months; however, these effects were probably associated with overall toxicity[46].

Reproductive toxicity: An increase in the rate of spontaneous abortions (15.9%) was reported among a group of 356 women who worked in a nickel hydrometallurgy refining plant in the Arctic region of Russia as compared to the rate (8.5%) in 342 local female construction workers[62].

Testicular degeneration was observed in rats and mice exposed to nickel sulphate ($\geq$1.4 mg Ni/m^3) and nickel subsulphide ($\geq$1.83 mg Ni/m^3 for rats and $\geq$3.65 mg Ni/m^3 for mice) 6 h/day for 12 days over a 16-day period[63-65]. In male rats exposed to NiSO$_4$ at 25 mg/kg, several testicular changes, such as interstitial cell proliferation, transport vessel walls, and reduced number of spermatozoa and some testicular enzymes such as steroid 3ß hydroxysteroid dehydrogenase were seen[106]. Das and Dasgupta[107] also reported decreased sperm count, sperm motility, and alteration of steroidogenesis in nickel treated rats. Nickel exposure elevates testicular lipid peroxidation and suppresses

antioxidant enzyme activities in rats[108]. Kakela *et al*[109] conducted a study to determine the reproductive effects of nickel in both female and male Wistar rats in which one or both genders were exposed to nickel at 10-100 ppm as $NiCl_2$ in drinking water. In males (exposed 28 or 42 days before copulation) $NiCl_2$ induced shrinkage of seminiferous tubules and decreased the number of spermatogonia in the tubules.

Carcinogenicity: Nickel (Ni^{+2}) compounds are potent carcinogens and can induce malignant transformation of rodent and human cells. Workers have been employed in nickel refinery facilities, nickel mining and smelting facilities, nickel alloy production facilities, stainless steel production facilities, nickel-cadmium battery production facilities, or as stainless steel welders are found to be suffering from cancer. Because nickel workers are exposed to several nickel species, it is difficult to assess the carcinogenic potential of a particular nickel species. The International Committee on Nickel Carcinogenesis in Man investigators used cross-classification analyses to examine the dose-response to a specific nickel species independent of variations in other species. The most comprehensive cross-classification analyses were performed for cohorts of workers in different departments at the Mond/INCO (Clydach) nickel refinery and at the Falconbridge (Kristiansand) nickel refinery (only analyzed for metallic nickel)[110]. A number of animal studies have examined the carcinogenic potential of nickel subsulphide, nickel oxide, and nickel sulphate. Chronic exposure to nickel subsulphide resulted in significant increases in lung tumours in two rat studies. Adenomas, adenocarcinomas, squamous cell carcinomas, and fibrosarcoma were observed in rats exposed to 0.7 mg Ni/m^3 as nickel subsulphide 6 h/day, 5 days/wk, for 78 wk[111]. Acute (6 h/day, 5 days/wk, for 1 month) inhalation exposure to $\leq$6.3 mg Ni/m^3 as nickel oxide resulted in no significant increase in lung cancer in rats $\leq$20 months after exposure[112]. However, significant increase in the incidence of alveolar/bronchiolar adenoma or carcinoma were observed in male and female rats exposed to 1 or 2 mg Ni/m^3 as nickel oxide 6 h/day, 5 days/wk for 2 yr[65]. The available evidence suggests that, mechanistically, nickel carcinogenicity is probably the result of genetic factors and/or direct (*e.g.*, conformational changes) or indirect (*e.g.*, generation of oxygen radicals) epigenetic factors. Additionally, certain nickel compounds promote cell proliferation, which would convert repairable DNA lesions into non repairable mutations.

Toxicities in some specific peripheral tissues

On liver: A transient increase in serum bilirubin was observed in 3 of 10 workers who were hospitalized after drinking water from a water fountain, contaminated with nickel sulphate[113]. In rats, decreased liver weight was observed following exposure for 28 days to 2 yr to 0.97-75 mg/kg/day of nickel chloride or nickel sulphate[43]. Recent studies on rats by Das *et al*[114] revealed a nickel sulphate-induced degenerative effect on hepatic tissue[114]. They have observed that after the intraperitoneal injection of nickel sulphate, normal hepatic architecture was greatly altered, along with appearance of vacuolated cytoplasm (fatty liver), eccentric nuclei, and Kupffer cell hypertrophy. One report described decreased hepatic and renal transaminase activities after nickel treatment in rats, which was found more deleterious in a protein-restricted dietary regimen[30]. Nickel sulphate also decreases the liver ascorbic acid and cholesterol levels in rats[115]. Das *et al*[88] showed, after the nickel treatment of rats, a significant rise in hepatic lipid peroxides and a decrease in antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities and in the hepatic glutathione concentration.

On lungs: Studies in both humans and animals indicate that following inhalation exposure, the respiratory system is the primary target of nickel toxicity. In humans, a single case of death from the ARDS occurred in a worker who sprayed a very high concentration of metallic nickel of small particle size for 90 min without wearing personal protective equipment[116]. The histological changes noted in the lungs of this case included alveolar wall damage with fibrotic changes and oedema in the alveolar space. A statistically significant increase in the incidence of deaths from respiratory disease was found in welders in some studies[117]. Studies in rats and mice demonstrate that chronic active inflammation in the lungs is the most prominent effect following inhalation exposure to nickel sulphate, nickel subsulphide, or nickel oxide. In acute exposure, however, chronic lung inflammation was observed at the lowest concentrations of nickel sulphate and nickel subsulphide[118]. Chronic exposure for 2 yr to nickel (6 h/day, 5 day/wk) resulted in active lung inflammation in mice at 0.06 mg Ni/m^3 and in rats at 0.11 mg Ni/m^3 and higher. Additional lung effects that were found at the same dose levels as those in inflammation include alveolar epithelium hyperplasia, fibrosis in rats and mice exposed to nickel subsulphide,

and bronchiolization and/or alveolar proteinosis in mice exposed to nickel oxide[119]. In addition to the lung effects in rats and mice, several studies have demonstrated that exposure to nickel sulphate and nickel subsulphide can induce atrophy of the nasal olfactory epithelium[63]. A recent study[120] revealed that water-soluble nickel sulphate has profound effect on lung tissue and its antioxidant enzyme system[119]. In this study, the intraperitoneal injection of nickel sulphate (2 mg/100 g b wt) alternatively for 10 days caused a significant increase in the lipid peroxide level in lung tissue in comparison with a control group. At the same time, lung SOD, CAT, and GSH-Px activities were significantly decreased[120].

On kidneys: Inflammation in the bronchioles, alveolar congestion, alveolar cell hyperplasia, and sometimes congestion in the lumen was also noticed[120]. Following oral exposure to nickel contaminated drinking water, a transient increase in urine albumin was reported in electroplating workers[37]. Increased levels of nickel in urine were significantly linked to urinary β_2-microglobulin levels in nickel refinery workers[121]. The kidney was the major organ of nickel accumulation, and minor renal tubular damage at the corticomedullary junction was observed[37]. In rats exposed for 13 wk to nickel sulphate in drinking water, a significant decrease in urine volume and urine glucose levels and an increase in relative kidney weight were also observed[45].

Nickel and oxidative stress

Heavy metals as free radical generators: The molecules of diatomic oxygen in the earth atmosphere are the biggest ones to provoke reactions on the living cells. Except for those organisms that are specially adapted to live under anaerobic conditions, all the animals and plants need oxygen for an efficient production of energy[122]. The oxygen emergency must have been followed by the ozone layer appearance (O_3) in the high atmosphere. The absorption of ultraviolet rays occurs due to change of ozone layer and probably it allowed the evolution of the most complex earth organism[122]. Lipid peroxidation sets off a chain reaction that generates a large numbers of free radicals, which are both cytotoxic and genotoxic through their ability to covalently modify proteins and DNA[123,124]. The mechanisms of heavy metals toxicity through electron transfer most often involve the cross-linking of the sulphhydryl groups of proteins. Nickel and other heavy metals can also generate free radicals directly from molecular oxygen in a two step process to produce superoxide anion. In the continued presence of the heavy metal, the superoxide anions formed can then combine with protons in the dismutation reaction generating hydrogen peroxide in the process.

Ni(II) induces oxidative stress: The cumulative production of reactive oxygen species/ reactive nitrogen species ROS/RNS through either endogenous or exogenous insults is termed oxidative stress and is common for many types of cancer cell that are linked with altered redox regulation of cellular signaling pathways. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells. The redox imbalance thus may be related to oncogenic stimulation. The ROS generated could non selectively damage DNA, possibly resulting in genetic changes in active genes. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions (8-OH-G) have been noted in various tumours, strongly implicating such damage in the aetiology of cancer[125]. Nickel may bind to DNA-repair enzymes and generate oxygen-free radicals to cause protein degradation *in situ*. This irreversible damage to the proteins involved in DNA repair, replication, recombination, and transcription could be important for the toxic effects of nickel[126].

Experimental data suggest that oxidative stress may be important in nickel-induced carcinogenesis, however, a direct correlation between the ability of nickel to produce oxidative stress and carcinogenicity is not yet fully understood. Nickel (Ni^{2+}) mimic hypoxia and was used as a tool to study the role of oxygen sensing and signaling cascades in the regulation of hypoxia-inducible gene expression. This metal can produce oxidative stress; therefore, it was conceivable that ROS may trigger signaling pathways resulting in the activation of the hypoxia-inducible factor (HIF)-1 transcription factor and upregulation of hypoxia-related genes. The activity of the HIF-1 transcription factor as assessed in transient transfection assays was stimulated by Ni^{2+}, hypoxia, and desferrioxamine, but this activation was not diminished when oxidative stress was attenuated nor was HIF-dependent transcription enhanced by hydrogen peroxide. It was reported that ROS are produced during the exposure of cells to metals that mimic hypoxia, but the formation of ROS was not involved in the activation of HIF-1-dependent genes[127]. HIF-1 is very sensitive to hypoxia stimulus and precise regulation of oxygen homeostasis. HIF-1 is composed of two bHLH proteins, HIF-1a and HIF-1b. HIF-1a is expressed and HIF-1b accumulated only in hypoxic cells. One explanation of the nickel-induced activation of the HIF-1 transcription

factor is based on the assumption that nickel replaces iron in the oxygen carrier, Fe(II)-hybrid haemoglobin. Substitution of iron by nickel switches signal to permanent hypoxia, which in turn activates the HIF-1 factor[127]. Ni(II) enhances the oxidation of all DNA bases *in vitro*[128] and lipid peroxidation *in vivo*[5]. Although nickel(II) by itself does not cause efficient free radical generation from oxygen, H_2O_2, or lipid hydroperoxides, the reactivity of Ni(II) with those oxygen derivatives can be modulated by chelation with certain histidine- and cysteine-containing ligands[129,130]. The incubation of Ni(II) with cysteine in an aerobic environment generates the hydroxyl radical, which then reacts with cysteine to generate a carbon-centered alkyl radical. Free radicals can also be generated from lipid hydroperoxides by Ni(II) in the presence of several oligopeptides[131,132]. Hence, free radical generation from the reaction of Ni(II)-thiol complexes and molecular oxygen, and/or lipid hydroperoxides, could play an important role in the mechanism(s) of Ni(II) toxicity[30]. The results of a series of studies using cultured human peripheral blood lymphocytes also suggest that nickel induces oxidative stress in humans[133,134]. In an *in vitro* study using human lymphocytes, nickel chloride increased the generation of hydrogen peroxide and lipid peroxidation[133]. The levels of intracellular reactive oxygen species, lipid peroxidation and hydroxyl radicals and also the potential effects of antioxidants were examined. The level of hydroxyl radical in the Ni-treated group was much higher than in control. Also the levels of thiobarbituric acid-reactive substances (TBARS) in human lymphocytes *in vitro* in a concentration-dependent manner were detected. Catalase partially reduced the $NiCl_2$- induced elevation of oxidants, whereas superoxide dismutase (SOD) enhanced the level of oxidants and TBARS. Both $NiCl_2$-induced lipid peroxidation was prevented significantly by glutathione (GSH) and mannitol. $NiCl_2$-induced increase in generation of hydroxyl radical was prevented significantly by catalase, GSH and mannitol, but not by SOD. These results suggest that $NiCl_2$-induced lymphocyte toxicity may be mediated by oxygen radical intermediates. Catalase, GSH and mannitol each provides protection against the oxidative stress induced by nickel[133].

The pretreatment of human blood lymphocytes with either CAT (a H_2O_2 scavenger), or SOD (a scavenger of O^{2-} radical) significantly reduced markers of nickel carbonate hydroxide-induced genetic and cellular damage. In another study, nickel chloride was shown to induce the free radicals-mediated induction of oxidized DNA bases and DNA-protein cross-links human lymphocytes *in vitro*[135]. $NiCl_2$-induced lymphocyte toxicity may be mediated by oxygen radical intermediates, for which the accelerated generation of OH may play an important role in nickel-induced oxidative damage of human lymphocytes[131]. In rats, the parenteral administration of nickel chloride enhances lipid peroxidation in liver, kidney, and lung, as measured by the thiobarbituric acid reaction for malondialdehyde (MDA) in fresh tissue homogenates[136]. The level of MDA was also found to be significantly elevated in serum of nickel chloride-treated rats[137]. Misra and coworkers showed that a single intraperitoneal injection of nickel (II) acetate increased lipid peroxidation and glutathione-S-transferase activity in rat liver and kidney while concomitantly decreasing the glutathione concentration and glutathione reductase activity[138]. The same group found that the nickel-induced lipid peroxidation in different strains of mice was concurrent with nickel's effect on antioxidant defense systems in liver and kidney[138,139]. The magnitude of nickel-induced lipid peroxidation showed a reverse correlation with the extent and direction of its effect on glutathione, glutathione peroxidase glutathione reductase, but not on CAT, SOD, or glutathione-S-transferase. Nickel chloride also induces lipid peroxidation in rat renal cortical slices *in vitro*[140].

Antioxidant defense against nickel toxicity: Suitable mechanisms are present in human body so that steady state concentration of potentially toxic oxygen derived free radicals are kept in check under normal physiological condition by body's intrinsic antioxidant defense system. But enhanced generation of these reactive oxygen species (ROS) can overwhelm cell's intrinsic antioxidant defenses and result in a condition known as "oxidative stress". Data suggest that antioxidants may play an important role in abating some hazards of nickel[141]. Exogenous antioxidants are intimately involved in the prevention of cellular damage by interacting with free radicals and by terminating the chain reaction. In rats, increased lipid peroxide formation and decreased levels of glutathione, SOD, CAT, GSH-Px activities as well as ascorbic acid depletion have been found in the most active metabolic tissues of the body, namely, liver and kidney[114,115]. Increased lipid peroxidation could stimulate phospholipase A_2 (PLA_2) activities, thereby causing the production of a variety of eicosanoids, products of the arachidonic acid pathway that are responsible for cell injury. Additionally, a decrease in antioxidant enzymes suggests an interaction with the accumulated free radicals

and active amino acids of the enzymes, leading to functional impairment and tissue damage. Ascorbic acid (vitamin C) is a dietary antioxidant that inactivates oxygen free radicals.

Some studies have shown that vitamin C works in concert with vitamin E to prevent the free radical chain oxidation of lipids. Numerous reports have shown the positive effect of vitamin C as an antioxidant and scavenger of free radicals[88]. Nickel compounds adversely affect cells by modulating ascorbic acid metabolism and other metabolic pathways. Reports have shown the positive effect of vitamin C as an antioxidant and scavenger of free radicals[88]. On the other hand, doses of vitamin C exceeding the daily recommended dietary allowance could lead to the formation of toxic advanced glycation end products, which are derived from the degradation products of carbohydrates, lipids, and ascorbic acid. Numerous animal studies support the ability of vitamin C to protect against free radical damage *in vitro*. One of our studies revealed that, L-ascorbic acid administration simultaneously with nickel sulphate improves nickel induced hyperglycaemia by boosting insulin sensitivity in rats and also improve liver glycogen content. The administration of L-ascorbic acid can improve the situation in rats by its scavenging activity within the lipid region of the membrane. Reports also showed that both the nickel-induced activation of hypoxia-inducible factor (HIF-1) and the upregulation of hypoxia-inducible genes are due to depleted intracellular ascorbate levels. The addition of ascorbate to the culture medium increased the intracellular ascorbate level and reversed both the metal-induced stabilization of HIF-1 and HIF-1α dependent gene expression[142]. α-tocopherol (vitamin E) can also protect against oxidative stress and improve nickel induced alteration of serum lipid profile and plasma glucose concentration. It is also reported that, α-tocopherol can protect both liver and pancreatic cells from nickel induced cellular damage. Vitamin E, situated near the cytochrome P-450 in the membrane phospholipid, sweeps the free radicals formed in the cytochrome P-450.

Nickel is a potent haematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic and carcinogenic agent. Although conflicting results have been reported in human studies about the effect of L-ascorbic acid and α-tocopherol supplementation on oxidative stress, the experimental animal results with ascorbic acid suggest that a high consumption of dietary L-ascorbic acid and α-tocopherol might ameliorate oxidative stress induced by nickel.

References

1. Nestle FO, Speidel H, Speidel MO. Metallurgy: High nickel release from 1- and 2-euro coins. *Nature* 2002; *419* : 132.

2. Hathaway K, Clark AE. *Magnetostrictive materials.* Warrendale, PA, USA: Materials Research Society; 1993. p. 34-41.

3. ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological profile for nickel.* Atlanta, GA, USA: ATSDR/ U.S. Public Health Service, ATSDR/TP-88/19; 1988.

4. Goyer R. Toxic effects of metals. In: Amdur MO, Doull JD, Klaassen CD, editors. *Casarett and Doull's toxicology*, 4th ed. New York: Pergamon Press; 1991. p. 623-80.

5. Coogan TP, Latta DM, Snow ET, Costa M. Toxicity and carcinogenicity of nickel compounds, In: McClellan RO, editor. *Critical reviews in toxicology*, vol. 19. Boca Raton, FL: CRC Press; 1989. p. 341-84.

6. Nielsen FH. Effect of form of iron on the interaction between nickel and iron in rats: Growth and blood parameters. *J Nutr* 1980; *110* : 965-73.

7. Ambrose AM, Larson PS, Borzelleca JR, Hennigar GR, Jr. Long term toxicologic assessment of nickel in rats and dogs. *J Food Sci Technol* 1976; *13* : 181-7.

8. Dieter MP, Jameson CW, Tucker AN, Luster MI, French JE, Hong HL, *et al*. Evaluation of tissue disposition, myelopoietic and immunologic responses in mice after long-term exposure to nickel sulphate in the drinking water. *J Toxicol Environ Health* 1988; *24* : 356-72.

9. Borg K, Tjalve H. Uptake of 63Ni²⁺ in the central and peripheral nervous system of mice after oral administration: Effects of treatment with halogenated 8-hydroxyquinolines. *Toxicology* 1989; *54* : 59-68.

10. Environmental Protection Agency. *Health assessment document for nickel and nickel compounds*, Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, EPA-600/8-83-012F. NTIS PB86-232212; 1986.

11. Bennett BG. Environmental nickel pathways in man. In: Sunderman FW Jr, editor. *Nickel in the human environment.* Proceedings of a joint symposium. IARC scientific publication no. 53. Lyon, France: International Agency for Research on Cancer; 1984. p. 487-95.

12. Grandjean P. Human exposure to nickel. In: Sunderman FW Jr, editor. *Nickel in the human environment.* Proceedings of a joint symposium, IARC scientific publication no. 53. Lyon, France: International Agency for Research on Cancer; 1984. p. 469-85.

13. Sunderman FW Jr, Oskarsson A. Nickel. In: Merian E, editor. *Metals and their compounds in the environment.* New York: VCH Verlagsgesellschaft; 1991. p. 1101-26.

14. Nielsen NH, Menné T, Kristiansen J, Christensen JM, Borg L, Poulsen LK. Effects of repeated skin exposure to low nickel concentrations: A model for allergic contact dermatitis to nickel on the hands. *Br J Dermatol* 1999; *141* : 676-82.

15. Patriarca M, Lyon TD, Fell GS. Nickel metabolism in humans investigated with an oral stable isotope. *Am J Clin Nutr* 1997; *66* : 616-21.

16. Sunderman FW Jr. Mechanisms of nickel carcinogenesis. *Scand J Work Environ Health* 1989; *15* : 1-12.

17. Fullerton A, Andersen JR, Hoelgaard A, Menné T. Permeation of nickel salts through human skin *in vitro*. *Contact Dermatitis* 1986; *15* : 173-7.

18. Norgaard O. Investigation with radioactive Ni-57 into the resorption of nickel through the skin in normal and in nickel-hypersensitive persons. *Acta Derm Venereol* 1955; *35* : 111-7.

19. Bernacki EJ, Parsons GE, Sunderman FW Jr. Investigation of exposure to nickel and lung cancer mortality. *Ann Clin Lab Sci* 1978; *8* : 190-4.

20. Elias Z, Mur JM, Pierre F, Gilgenkrantz S, Schneider O, Baruthio F, *et al*. Chromosome aberrations in peripheral blood lymphocytes of welders and characterization of their exposure by biological samples analysis. *J Occup Med* 1989; *31* : 477-83.

21. Angerer J, Lehnert G. Occupational chronic exposure to metals. II: Nickel exposure of stainless steel welders-biological monitoring. *Int Arch Occup Environ Health* 1990; *62* : 7-10.

22. Kollmeier H, Seemann JW, Muller KM. Increased chromium and nickel content in lung tissue and bronchial carcinoma. *Am J Ind Med* 1987; *11* : 659-69.

23. Raithel HJ, Schaller KH, Reith A, Svenes KB, Valentin H. Investigations on the quantitative determination of nickel and chromium in human lung tissue industrial medical toxicological and occupational medical expertise aspects. *Int Arch Occup Environ Health* 1988; *60* : 55-66.

24. Andersen I, Svenes KB. Determination of nickel in lung specimens of thirty-nine autopsied nickel workers. *Int Arch Occup Environ Health* 1989; *61* : 289-95.

25. Whanger PD. Effects of dietary nickel on enzyme activities and mineral content in rats. *Toxicol Appl Pharmacol* 1973; *25* : 323-31.

26. EPA. *Drinking water regulations and health advisories*. Washington DC: U.S. Environmental Protection Agency, Office of Water; 1996. EPA822R96001.

27. Sunderman FW Jr, Hopfer SM, Sweeney KR, Marcus AH, Most BM, Creason J. Nickel absorption and kinetics in human volunteers. *Proc Soc Exp Biol Med* 1989; *191* : 5-11.

28. Sunderman FW Jr, Aito A, Morgan LG, Norseth T. Biological monitoring of nickel. In: Nieboer E, Nriagu JO, editors. *Nickel and human health*. New York: John Wiley and Sons, Inc.; 1986. p. 49-68.

29. Sunderman FW Jr, Hopfer SM, Cristomo MC, Stoeppler M. Rapid analysis of nickel in urine by electrothermal atomic absorption spectrophotometry. *Ann Clin Lab Sci*. In: Nieboer E, Nriagu JO, editors. *Nickel and human health*. New York: John Wiley and Sons, Inc.; 1986. p. 49-68.

30. Das KK, Buchner V. Effect of nickel exposure on peripheral tissues: Role of oxidative stress in toxicity and possible protection by ascorbic acid. *Rev Environ Health* 2007; *22* : 133-49.

31. IARC (International Agency for Research on Cancer). *IARC Monograph on the evaluation of carcinogenic risks to humans*. vol. 49. Lyans, France: IARC; 1990. p. 318-411.

32. U.S. Department of Health and Human Services (DHHS). *Seventh annual report on carcinogens: Summary 1994*. Research Triangle Park, NC, USA: DHHS, National Institute of Environmental Health Sciences; 1994. p. 262-9.

33. *http:\\www.enia.org/index.cfm/ci_id/3741.htm*, accessed on February 2, 2008.

34. Sunderman FW Jr, Coulston F, Eichhorn GL. *Nickel*. Washington DC: National Academy of Science; 1975. p. 97-143.

35. Daldrup T, Haarhoff K, Szathmary SC. Toedliche nickel sulfaye-intoxikation. *Berichte zur Serichtlichen Medizin* 1983; *41* : 141-4.

36. Rendall REG, Phillips JI, Renton KA. Death following exposure to fine particulate nickel from a metal arc process. *Ann Occup Hyg* 1994; *38* : 921-30.

37. Sunderman FW Jr, Dingle B, Hopfer SM, Swift T. Acute nickel toxicity in electroplating workers who accidentally ingested a solution of nickel sulphate and nickel chloride, *Am J Ind Med* 1988; *14* : 257-66.

38. Clemons G, Garcia JF. Neuroendocrine effects of acute nickel chloride administration in rats. *Toxicol Appl Pharmacol* 1981; *61* : 343-8.

39. Kasprzak K, Gabryel P, Jarezewska K. Nickel toxicology. *Proc Int Conference* 1980; *2* : 59-62.

40. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological profile for Nickel*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2005. p. 95-107.

41. Akesson B, Skervfing S. Exposure in welding of high nickel alloy. *Int Arch Occup Environ Health* 1985; *56* : 111-7.

42. Weber CW, Reid BL. Nickel toxicity in young growing mice. *J Anim Sci* 1969; *28* : 620-3.

43. Weischer CH, Kordel W, Hochrainer D. Effects of $NiCl_2$ and NiO in Wistar rats after oral uptake and inhalation exposure, respectively. *Zentral Bakteriol Mikrobiol Hyg* (B) 1980; *171* : 336-51.

44. IRIS. *Integrated risk information system*. Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency; 1996.

45. Obone E, Chakrabarty SK, Bai C, Malick MA, Lamantagne L, Subramanian KS. Toxicity and biaoaccumulation of nickel sulphate in Sprague-Dawley rats following 13 weeks of subchronic exposure. *J Toxicol Environ Health* 1999; *57*: 379-401.

46. American Biogenics Corporation. *Ninety day gavage study in albino rats using nickel*. Final report submitted to U.S. Environmental Protection Agency, Office of Solid Waste. Study 410-2520. Submitted by Research Triangle Institute and American Biogenics Corporation; 1988.

47. Vyskocil A, Senft V, Viau C, Cízková M, Kohout J. Biochemical renal changes in workers exposed to soluble nickel compounds. *Hum Exp Toxicol* 1994; *13* : 257-61.

48. Wall LM, Calnan CD. Occupational nickel dermatitis in the electroforming industry. *Contact Dermatitis* 1980; *6* : 414-20.

49. Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, *et al*. Toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulphate, or nickel sulphate

hexahydrate in F344/N rats and B6C3F$_1$ mice. *Fundam Appl Toxicol* 1989; *12* : 584-94.

50. Haley PJ, Shopp GM, Benson JM, Cheng YS, Bice DE, Luster MI, *et al*. The immunotoxicity of three nickel compounds following 13-week inhalation exposure in the mouse. *Fundam Appl Toxicol* 1990; *15* : 476-87.

51. Smialowicz RJ, Rogers RR, Riddle MM, Scott GA. Immunologic effects of nickel: I. Suppression of cellular and humoral immunity. *Environ Res* 1984; *33* : 413-27.

52. Smialowicz RJ, Rogers RR, Riddle MM, Garner RJ, Rowe DG, Luebke RW. Immunologic effects of nickel: II. suppression of natural killer cell activity. *Environ Res* 1985; *36* : 56-66.

53. Smialowicz RJ, Rogers RR, Rowe DG, Riddle MM, Luebke RW. The effects of nickel on immune function in the rat. *Toxicology* 1987; *44* : 271-81.

54. Adkins B Jr, Richards JH, Gardner DE. Enhancement of experimental respiratory infection following nickel inhalation. *Environ Res* 1979; *20* : 33-42.

55. Haley PJ, Bice DE, Muggenburg BA, Hahn FF, Benjamin SA. Immunopathologic effects of nickel subsulphide on the primate pulmonary immune system. *Toxicol Appl Pharmacol* 1987; *88* : 1-12.

56. United State Air Force. Nickel. In: Harry G, editor. *Installation restoration program toxicology guide*, vol. 5. Wright Patterson AFB, OH: Armstrong Aerospace Medical Research Laboratory; 1990.

57. NAS. *Nickel*. National Academy of Sciences. Washington, DC: National Academy Press; 1975. p. 4, 5, 17.

58. Enterline PE, Marsh GM. Mortality among workers in a nickel refinery and alloy plant in West Virginia. *J Natl Cancer Inst* 1982; *68* : 925-33.

59. Wehner AP. Health and environmental effects of aerosols: Biological effects and fate of inhaled man-made and natural aerosols in animal models. *J Aerosol Sci* 1986; *17* : 305-15.

60. Schroeder HA, Mitchener M, Nason AP. Life-term effects of nickel in rats: Survival, tumors, interactions with trace elements and tissue levels. *J Nutr* 1974; *104* : 239-43.

61. Warner JS. Nickel carbonyl. Prenatal exposure. *Science* 1979; *203* : 1194-5.

62. Chashschin VP, Artunina GP, Norseth T. Congenital defects, abortion and other health effects in nickel refinery workers. *Sci Total Environ* 1994; *148* : 287-91.

63. National Toxicology Programme. *NTP technical report on the toxicology and carcinogenesis studies of nickel oxide (CAS No. 1313-99-1) in F344/N Rats and B6C3F1 Mice (inhalation studies)*. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NTP TRS No. 451; 1996.

64. National Toxicology Programme. *NTP technical report on the toxicology and carcinogenesis studies of nickel subsulphide (CAS No. 12035-72-2) in F344/N Rats and B6C3F1 Mice (inhalation studies)*. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NTP-TRS No. 453; 1996.

65. National Toxicology Programme. *NTP technical report on the toxicology and carcinogenesis studies of nickel sulphate hexahydrate (CAS No. 10101-97-0) in F344/N Rats and B6C3F1 Mice (inhalation studies)*. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public

Health Service, National Institutes of Health. NTP-TRS No. 454; 1996.

66. Costa M. Molecular mechanism of nickel carcinogenesis. *Annu Rev Pharmacol Toxicol* 1991; *3* : 321-37.

67. Das KK, Dasgupta S. Effect of nickel on testicular nucleic acid concentrations of rats on protein restriction. *Biol Trace Elem Res* 2000; *73* : 175-80.

68. Waksvik H, Boysen M. Cytogenic analysis of lymphocytes from workers in a nickel refinery. *Mutat Res* 1982; *103* : 185-90.

69. Andersen O. Effects of coal combustion products and metal compounds on sister chromatid exchange (SCE) in a macrophage cell line. *Environ Health Perspect* 1983; *47* : 239-53.

70. Hansen K, Stern RM. Toxicity and transformation potency of nickel compounds in BHK cells *in vitro*. In: Sunderman FW Jr, Aitio A, Berlin A, editors. *Nickel in the human environment*. IARC scientific publication no. 53. Lyon, France: International Agency for Research on Cancer; 1984. p. 193-200.

71. Biedermann KA, Landolph JR. Induction of anchorage independence in human diploid foreskin fibroblasts by carcinogenic metal salts. *Cancer Res* 1987; *47* : 3815-23.

72. Conway K, Costa M. Nonrandom chromosomal alterations in nickel-transformed Chinese hamster embryo cells. *Cancer Res* 1989; *49* : 6032-8.

73. Arlauskas A, Baker RS, Bonin AM, Tandon RK, Crisp PT, Ellis J. Mutagenicity of metal ions in bacteria. *Environ Res* 1985; *36* : 379-88.

74. Biggart NW, Costa M. Assessment of the uptake and mutagenicity of nickel chloride in *Salmonella* tester strains. *Mutat Res* 1986; *175* : 209-15.

75. Wong PK. Mutagenicity of heavy metals. *Bull Environ Contam Toxicol* 1988; *40* : 597-603.

76. Ogawa HI, Shibahara T, Iwata H, Okada T, Tsuruta S, Kakimoto K, *et al*. Genotoxic activities *in vivo* of cobaltous chloride and other metal chlorides as assayed in the *Drosophila* wing spot test. *Mutat Res* 1994; *320* : 133-40.

77. Hartwig A, Beyersmann D. Enhancement of UV-induced mutagenesis and sister-chromatidexchanges by nickel ions in V79 cells: Evidence for inhibition of DNA repair. *Mutat Res* 1989; *217* : 65-73.

78. Hsie AW, Johnson NP, Couch DB. Quantitative mammalian cell mutagenesis and apreliminary study of the mutagenic potential of metallic compounds. In: Kharasch N, editor. *Trace metals in health and disease*. New York: Raven Press; 1979. p. 55-69.

79. Fletcher GG, Rossetto FE, Turnbull JD, Nieboer E. Toxicity, uptake, and mutagenicity of particulate and soluble nickel compounds. *Environ Health Perspect* 1994; *102* (Suppl 3): 69-79.

80. Amacher DE, Paillet SC. Induction of trifluorothymidine resistant mutants by metal ions in L5178Y/TK+/- cells. *Mutat Res* 1980; *78* : 279-88.

81. McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, *et al*. Responses of the L5178Y TK+/TK- mouse lymphoma cell forward mutation assay. III 72 coded chemicals. *Environ Mol Mutagen* 1988; *12* : 85-154.

82. Biggart NW, Murphy E Jr. Analysis of metal-induced mutations altering the expression or structure of a retroviral gene in a mammalian cell line. *Mutat Res* 1988; *198* : 115-30.

83. Biggart NW, Gallick GE, Murphy EC Jr. Nickel-induced heritable alterations in retroviral transforming gene expression. *J Virol* 1987; *61* : 2378-88.

84. Mayer C, Klein RG, Wesch H, Schmezer P. Nickel subsulphide is genotoxic *in vitro* but shows no mutagenic potential in respiratory tract tissues of BigBlue (TM) rats and Muta (TM) Mouse mice *in vivo* after inhalation. *Mutat Res* 1998; *420* : 85-98.

85. Hartwig A, Kruger I, Beyersmann D. Mechanisms in nickel genotoxicity: The significance of interactions with DNA repair. *Toxicol Lett* 1994; *72* : 353-8.

86. Lee-Chen SF, Yu CT, Wu DR, Jan KY. Differential effects of luminol, nickel and arsenite on the rejoining of ultraviolet light and alkylation-induced DNA breaks. *Environ Mol Mutagen* 1994; *23* : 116-20.

87. Lynn S, Yew FH, Chen KS, Jan KY. Reactive oxygen species are involved in nickel inhibition of DNA repair. *Environ Mol Mutagen* 1997; *29* : 208-16.

88. Das KK, Das SN, Dasgupta S. The influence of ascorbic acid on nickel induced hepatic lipid peroxidation in rats. *J Basic Clin Physiol Pharmacol* 2001; *12* : 187-94.

89. Chashschin VP, Artunina PA, Norseth T. Congenital defects, abortion and other health effects in nickel refinery workers. *Science Total Environ* 1994; *148* : 287-91.

90. Weischer CH, Kordel W, Hochrainer D. Effects of $NiCl_2$ and NiO in Wistar rats after oral uptake and inhalation exposure, respectively. *Zentral Bakteriol Mikrobiol Hyg* (B) 1980; *171* : 336-51.

91. Chernoff N, Kavlock RJ. An *in vivo* teratology screen utilizing pregnant mice. *J Toxicol Environ Health* 1982; *10* : 541-50.

92. Käkelä R, Käkelä A, Hyvarinen H. Effects of nickel chloride on reproduction of the rat and possible antagonistic role of selenium. *Comp Biochem Physiol C* 1999; *123* : 27-33.

93. Berman E, Rehnberg B. *Fetotoxic effects of nickel in drinking water in mice*. National Technical Information Service. EPA600183007. PB83225383; Virginia USA: Spring Field; 1983.

94. Research Triangle Institute. *Two-generation reproduction and fertility study of nickel chloride administered to CD rats in the drinking water: Fertility and reproductive performance of the Po generation*. Final study report (II of III). Research Triangle Park, NC: Office of Solid Waste Management, U.S. Environmental Protection Agency; 1988.

95. Research Triangle Institute. *Two-generation reproduction and fertility study of nickel chloride administered to CD rats in the drinking water: Fertility and reproductive performance of the F1 generation*. Final study report (III of III). Research Triangle Park, NC: Office of Solid Waste Management, U.S. Environmental Protection Agency; 1988.

96. Das KK, Gupta AD, Dhundasi SA, Patil AM, Das SN, Ambekar JG. Protective role of L-ascorbic acid on antioxidant defense system in erythrocytes of albino rats exposed to nickel sulphate. *Biometals* 2007; *20* : 177-84.

97. Hostynek JJ. Sensitization to nickel: etiology, epidemiology, immune reactions, prevention, and therapy. *Rev Environ Health* 2006; *21* : 253-80.

98. Kimber I, Dearman RJ. Immune responses to contact and respiratory allergens. In: Dean JH, Luster MI, Munson AE, Kimber I, editors. *Immunotoxicology and Immunopharmacology*. New York, USA: Raven Press, Ltd.; 1994. p. 663-79.

99. Dearman J, Kimber I. Divergent immune responses to respiratory and contact chemical allergens: antibody elicited by phthalic anhydride and oxazolone. *Clin Exp Allergy* 1992; *22* : 241-50.

100. Guimaraens D, Gonzales MA, Condé–Salazar L. Systemic contact dermatitis from dental crowns. *Contact Dermatitis* 1994; *30* : 124-5.

101. Bencko V, Wagner V, Wagnerová M, Reichrtová E. Immuno-biochemical findings in groups of individuals occupationally and nonoccupationally exposed to emissions containing nickel and cobalt. *J Hyg Epidemiol Microbiol Immunol* 1983; *27* : 387-94.

102. Bencko V, Wagner V, Wagnerová M, Zavázal V. Human exposure to nickel and cobalt: Biological monitoring and immunobiological response. *Environ Res* 1986; *40* : 399-410.

103. Graham JA, Miller FJ, Daniels MJ, Payne EA, Gardner DE. Influence of cadmium, nickel and chromium on primary immunity in mice. *Environ Res* 1978; *16* : 77-8.

104. Figoni RA, Treagan L. Inhibitory effect of nickel and chromium upon antibody response of rats to immunization with T-1 phage. *Res Commun Chem Pathol Pharmacol* 1975; *11* : 335-8.

105. Schiffer RB, Sunderman FW Jr, Baggs RB, Moynihan JA. The effects of exposure to dietary nickel and zinc upon humoral and cellular immunity in SJL mice. *Neuroimmunol* 1991; *34* : 229-39.

106. EPA (US Environmental Protection Agency). *Drinking water criteria document for nickel*. NTIS PB86-117801. Cincinnati, OH: Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency; 1985.

107. Das KK, Dasgupta S. Effect of nickel sulphate on testicular steroidogenesis in rats during protein restriction. *Environ Health Perspect* 2002; *110* : 923-6.

108. Gupta AD, Dhundasi SA, Ambekar JG, Das KK. Effect of L-ascorbic acid on antioxidant defense system in testes of albino rats exposed to nickel sulphate. *J Basic Clin Physiol Pharmacol* 2007; *18* : 87-95.

109. Kakela RA, Kakela A, Hyvarinen H. Effects of nickel chloride on reproduction of the rat and possible antagonistic role of selenium. *Comp Biochem Physiol Pharmacol Toxicol Endocrinol* 1999; *123* : 27-37.

110. International Committee on Nickel Carcinogenesis in Man. Report of the International Committee on Nickel Carcinogenesis in Man. *Scand J Work Environ Health* 1990; *16* : 1-82.

111. Ottolenghi AD, Haseman JK, Payne WW, Falk HL, MacFarlnad HN. Inhalation studies of nickel sulphide in pulmonary carcinogenesis of rats. *J Natl Cancer Inst* 1974; *54* : 1165-72.

112. Horie A, Tanaka I, Haratake J. Electron microscopy of pulmonary lesions including carcinoma, induced by inhalation exposure of rats to nickel oxide aerosol. In: Brown SS, Sunderman FW Jr, editors. *Progress in nickel toxicology*. Proceedings of the 3rd International Congress on Nickel Metabolism and Toxicology. Oxford, UK: Blackwell; 1985. p. 41-4.

113. Sunderman FW Jr, Dingle B, Hopfer SM, Swift T. Acute nickel toxicity in electroplating workers who accidently ingested a solution of nickel sulfate and nickel chloride. *Am J Ind Med* 2007; *14* : 257-66.

114. Das KK, Gupta AD, Dhundasi SA, Patil AM, Das SN, Ambekar JG. Effect of L-ascorbic acid on nickel-induced alterations in serum lipid profiles and liver histopathology of rats. *J Basic Clin Physiol Pharmacol* 2006; *17* : 29-44.

115. Das KK, Dasgupta S. Studies on the role of nickel in the metabolism of ascorbic acid and cholesterol in experimental animals. *Ind J Physiol Allied Sci* 1998; *52* : 58-62.

116. Rendall REG, Phillips JI, Renton KA. Death following exposure to fine particulate nickel from a metal arc process. *Ann Occup Hyg* 1994; *38* : 921-30.

117. Cornell RG, Landis JR. Mortality patterns among nickel/ chromium alloy foundry workers. In: Sunderman FW Jr, Aitio A, Berlin A, editors. *Nickel in the human environment*. IARC scientific publication no. 53. Lyon, France: International Agency for Research on Cancer; 1984. p. 87-93.

118. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Nickel. U.S. Department of Health and Human Services, Public Health Service, ATSDR. Atlanta, Georgia, USA: U.S. Government Printing Office; 2003. p. 5-16.

119. National Toxicology Programme. NTP Toxicology and Carcinogenesis Studies of Nickel Oxide (CAS No. 1313-99-1) in F344 Rats and B6C3F1 Mice (Inhalation Studies). *Natl Toxicol Program Tech Rep Ser* 1996; *451* : 1-381.

120. Gupta, AD, Patil AM, Ambekar JG, Das SN, Dhundasi SA, Das KK. L-ascorbic acid protects the antioxidant defense system in nickel-exposed albino rat lung tissues. *J Basic Clin Physiol Pharmacol* 2006; *17* : 87-100.

121. Sunderman FW Jr, Horak E. Biochemical indices of nephrotoxicity, exemplified by studies of nickel nephropathy. In: Brown SS, Davies DS, editors. *Organ directed toxicity: Chemical indices and mechanisms*. London, U.K: Pergmon Press; 1981. p. 52-64.

122. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine.* Oxford: Clarendon Press; 1989. p. 543.

123. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malon-aldehyde and related aldehydes. *Free Radic Biol Med* 1991; *11* : 81-128.

124. Benedetti A, Comporti M, Esterbauer H. Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. *Biochim Biophys Acta* 1980; *620* : 281-96.

125. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006; *160* : 1-40.

126. Lynn S, Yew FH, Chen KS, Jan KY. Reactive oxygen species are involved in nickel inhibition of DNA repair. *Environ Mol Mutagen* 1998; *29* : 208-16.

127. Salnikow K, Su W, Blagosklonny MV, Costa M. Carcinogenic metals induce hypoxia-inducible factor-stimulated transcription by reactive oxygen species-independent mechanism. *Cancer Res* 2000, *60* : 3375-8.

128. Salnikow K, Gao M, Voitkun V, Huang X, Costa M. Altered oxidative stress responses in nickel-resistant mammalian cells. *Cancer Res* 1994; *54* : 6407-12.

129. Shi X, Dalal NS, Kasprzak KS. Generation of free radicals in reactions of Ni (II)-thiol complexes with molecular oxygen and model lipid hydro-peroxides. *J Inorg Biochem* 1993; *50* : 211-25.

130. Shi X, Dalal NS, Kasprzak KS. Generation of free radicals from lipid hydroperoxides by Ni^{2+} in the presence of oligopeptides. *Arch Biochem Biophys* 1992; *299* : 154-62.

131. Chen CY, Huang YF, Lin YH, Yen SF. Nickel-induced oxidative stress and effect of antioxidants in human lymphocytes. *Arch Toxicol* 2003; *77* : 123-30.

132. Chen CY, Huang YF, Huang WR, Huang YT. Nickel induces oxidative stress and genotoxicity in human lymphocytes. *Toxicol Appl Pharmacol* 2003; *189* : 153-9.

133. M'Bemba-Meka P, Lemieux N, Chakrabarti SK. Role of oxidative stress, mitochondrial membrane potential, and calcium homeostasis in nickel sulphate-induced human lymphocyte death *in vitro. Chem Biol Interact* 2005; *156* : 69-80.

134. M'Bemba-Meka P, Lemieux N, Chakrabarti SK. Role of oxidative stress and intracellular calcium in nickel carbonate hydroxide-induced sister-chromatid exchange, and alterations in replication index and mitotic index in cultured human peripheral blood lymphocytes. *Arch Toxicol* 2007; *81* : 89-99.

135. Wozniak K, Blasiak J. Free radicals-mediated induction of oxidized DNA bases and DNA-protein cross-links by nickel chloride. *Mutat Res* 2002; *514* : 233-43.

136. Sunderman FW Jr, Marzouk A, Hopfer SM, Zaharia O, Reid MC. Increased lipid peroxidation in tissues of nickel chloride-treated rats. *Ann Clin Lab Sci* 1985; *15* : 229-36.

137. Chen CY, Huang YL, Lin YH. Association between oxidative stress and cytokine production in nickel-treated rats. *Arch Biochem Biophys* 1998; *356* : 127-32.

138. Misra M, Rodriguez RE, Kasprzak KS. Nickel induced lipid peroxidation in the rat: correlation with nickel effect on antioxidant defense systems. *Toxicology* 1990; *64* : 1-17.

139. Rodriguez RE, Misra M, North SL, Kasprzak KS. Nickel-induced lipid peroxidation in the liver of different strains of mice and its relation to nickel effects on antioxidant systems. *Toxicol Lett* 1991; *57* : 269-81.

140. Chakrabarti SK, Bai C. Role of oxidative stress in nickel chloride-induced cell injury in rat renal cortical slices. *Biochem Pharmacol* 1999; *58* : 1501-10.

141. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal induced oxidative damage. *Curr Top Med Chem* 2001; *1* : 529-39.

142. Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM, Kasprzak KS. Depletion of intracellular ascorbate by the carcinogenic metal nickel and cobalt results in the induction of hypoxic stress. *J Biol Chem* 2004; *279* : 40337-44.

Reprint requests: Dr Kusal K. Das, Professor, Environmental Health Research Unit, Department of Physiology
Al Ameen Medical College, Bijapur 586 108, Karnataka, India
e-mail: kusaldas@yahoo.com