

## Effect of Nickel Exposure on Peripheral Tissues: Role of Oxidative Stress in Toxicity and Possible Protection by Ascorbic Acid

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**Abstract:** The vast industrial use of nickel has led to environmental pollution by the metal and its by-products during production, recycling, and disposal. Nickel is a known hematotoxic, immunotoxic, hepatotoxic, pulmonary toxic, and nephrotoxic agent. Allergic skin reactions are common in individuals who are sensitive to nickel. This article presents a selective review on nickel and its effect on certain metabolically active peripheral tissues of human and animals. The subtopics include nickel sources and uses, exposure pathways, transport, excretion, general health effects, and specific acute and chronic nickel toxicities in peripheral tissues like liver, lungs, and kidneys. The review particularly addresses the nickel-induced generation of reactive oxygen species and increased lipid peroxidation in various metabolically active tissues in humans and animals, and the possible role of vitamin c as a protective antioxidant.

**Keywords:** heavy metals, hemotoxicity, hepatotoxicity, pulmonary toxicity, nephrotoxicity, lipid peroxidation

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### INTRODUCTION

The twenty first century has brought an overall crisis for civilization. The rapid growth of science and technology has led to increased environmental disorder resulting in pollution. In developing countries, natural resources are squeezed out to their maximum limits with total disregard for environmental safety /1-2/. The most dangerous situation arises from the passage of pollutants and environmental hazards beyond boundaries. The heavy metal nickel (Ni) is a potentially toxic element, and as such, merits special consideration.

The properties of nickel and its environmental distribution have been summarized by the US Agency for Toxic Substances and Disease Registry /2/. Briefly, pure nickel is a silver-white, lustrous, hard, malleable, ductile, ferromagnetic metal that

is relatively resistant to corrosion and is a fair conductor of heat and electricity. These properties makes it desirable for use with other metals to form alloys, for example, iron (Fe), copper (Cu), chromium (Cr), and zinc (Zn). Nickel is used mainly for producing stainless steel. In the environment, nickel is primarily found combined with oxygen or sulfur as oxides or sulfides 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.

The vast industrial use of the metal has led to widespread environmental pollution during its production, recycling, and disposal. Nickel is released into atmosphere during nickel mining and by industries such as oil-burning power plants, coal-burning power plants, trash incinerators, rubber, and plastic industries, nickel-cadmium

battery industries, and electroplating industries. Nickel emitted from the stacks of power plants attaches to small particles of dust. Subsequently, the particles settle to the ground or wash out in rain or snow. Nickel is also released from industrial wastewater into soil or sediment where it strongly attaches to particles containing Fe or manganese (Mn) /2/.

### Environmental Exposure

The predominant sources of nickel exposure are food, breathing air, drinking water, tobacco smoking, skin contact with metal-plated nickel, nickel coins, stainless steel, jewelry, and artificial body parts.

*Water sources.* Nickel can enter groundwater and surface waters via dissolved rocks and soils, biological cycles, atmospheric fallout, industrial processes, waste disposal, leaching from dump sites, and acid rain, which mobilizes nickel from soil and increases groundwater concentrations. Drinking water usually contains less than 20 µg of nickel per L, but much higher concentrations could occur due to leaching from nickel-containing pipes and nickel-plated faucets or pollution of water supplies.

The level of nickel in river and lake waters is often as low as 10 parts per billion (ppb) or less (except in industrial areas). Although normal drinking water contains only 2 to 4.3 ppb nickel, levels as high as 72 ppb have been found in drinking water near areas of a large natural nickel deposit where nickel is mined and refined.

*Air sources.* Nickel can enter the atmosphere from natural sources like volcanic emissions and windblown dusts produced by the weathering of rocks and soils, combustion of fossil fuels, emissions of mining and refining operations, metal consumption in industrial processes, and waste incineration. Substantial concentrations of nickel in the atmosphere derive from the release of fly-ash from coal-fired power plants, and petroleum-derived nickel is released in automotive exhaust

fumes.

Nickel is present at very low levels in the environment and in air is attached to small particles. In the United States between 1977 and 1982, average nickel concentrations in air ranged from 7 to 12 ng/m<sup>3</sup> /2/. Subsequently, the U.S. Environmental Protection Agency (EPA) estimated that the average nickel concentration in air in the U.S. decreased to 2.2 ng/m<sup>3</sup>, based on air quality information obtained from 1996 /3/. Cigarette smoking can increase the amount of inhaled nickel by as much as 4 µg per pack of cigarettes.

The Threshold Limit Value (TLV), as defined by the American Conference of Governmental Industrial Hygienists (ACGIH), is the concentration of a substance to which most workers can be exposed without adverse effect /4/. The TLV can be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL). The TLV-TWA for air differs among different nickel compounds as well as among different agencies. The ACGIH increased the 8-h TLV-TWA for nickel carbonyl from 0.001 to 0.05 ppm /5/, whereas NIOSH still recommends the 0.01 ppm value /6/. Soluble nickel compounds have ACGIH 8-h TLV-TWA of 0.1 mg/m<sup>3</sup>.

*Other sources.* Wearing or handling jewelry, coins, and utensils that are fabricated from nickel alloys or that have nickel-plated coatings can induce allergic dermatitis in nickel-sensitive individuals. The implantation of nickel-containing prostheses or the administration of nickel-contaminated medications can lead to acute toxicity and immunologic disturbances resulting from parenteral exposure. Soil usually contains 4 to 80 parts per million (ppm) of nickel, with concentration of up to 9,000 ppm occurring near industries that extract nickel from ore /2/. Exposure to nickel in soil is normally through skin contact.

Oral environmental exposure of humans to high levels of soluble nickel compounds is extremely unlikely. Nevertheless, children who have an abnormal craving or appetite (pica) for nonfood substances, such as dirt, paint, or clay can be

exposed to nickel by eating soil. In the general population, food is generally the predominant oral source of nickel exposure. Nickel intake through food is about 170  $\mu\text{g/day}$  /2/. Foods such as tea, coffee, chocolate, soybeans, nuts, oatmeal, cabbage, spinach, potatoes, contain high concentrations of nickel.

Occupational exposure can occur in industries that process or use nickel. Breathing dust or fumes (as from welding) or by skin contact with nickel-containing metal and dust or solutions containing dissolved nickel compounds are the most common forms of occupational exposure /2/. Sulfide ores are processed by roasting, smelting, and converting, during which sulfur and iron are removed to yield a sulfur-deficient copper-nickel matte consisting primarily of nickel subsulfide. In sulfide roasting, the TLV is 1  $\text{mg/m}^3$  for nickel fume and dust.

### Nickel Absorption and Transport

Nickel enters the body when breathing air containing nickel, drinking water or eating food that contains nickel, or when skin comes into contact with nickel. Contrary to insoluble nickel compounds, the soluble salts of nickel are readily absorbed by the pulmonary and digestive tracts, and less by the skin. Inhaled nickel carbonyl is rapidly absorbed by the lungs and enters red blood cells where the compound undergoes conversion to  $\text{Ni}^{2+}$  and carbon monoxide. In nickel refinery workers, some inhaled nickel was slowly absorbed, as evidenced by sustained hypernickemia many years after cessation of exposure. During hemodialysis, traces of  $\text{Ni}^{2+}$  in hemodialysis fluid can be absorbed into the plasma, owing to the chelating action of plasma albumin. Oral or parenteral exposures to nickel can potentiate hand eczema in nickel-sensitive persons.

Only the small nickel particles in air can enter deep into the lungs. The volatility and lipid solubility of nickel carbonyl enable it to cross cell membranes. In tissue cytosol, nickel is bound to

several proteins and peptides. A small amount of nickel can enter the blood-stream from skin contact. In human plasma,  $\text{Ni}^{2+}$  is transported in blood bound to albumin or histidine or to a plasma metalloprotein called nickeloplasmin /2,7/. Mean nickel concentrations found in human tissues in descending order are lung > thyroid > adrenal gland > kidney > heart > liver > brain > spleen > pancreas.

### Nickel Excretion

Nickel is not a cumulative toxicant and most of the absorbed metal is excreted rapidly. In humans, nickel in the bloodstream leaves in the urine. Most ingested nickel leaves quickly in the feces, but a small amount can enter the blood and leave in the urine. /2, 7/. According to Sunderman et al /7/, serum and urine nickel levels are the most useful biomarkers of nickel exposure. In the general population, the average nickel concentrations in serum and urine are 0.2  $\mu\text{g/L}$  and 0.1 to 13.3  $\mu\text{g/L}$ , respectively /8-9/. A significant correlation was found between occupational exposure to the less-soluble nickel compounds and the level of nickel in urine in various groups of workers/10/. Bernacki et al /11/ found a relation between the exposure concentration of soluble nickel compounds and the level of nickel in urine. In a study by Angerer et al /9/, nickel in the urine of welders was found at concentrations > 18.8  $\mu\text{g/L}$ .

The urinary halftime to nickel via inhalation varies from 17 to 39 h, whereas only 8 h is required following oral intake. Nickel is also excreted in sweat (at a concentration 10 to 20 times higher than in urine), through the hair, and via human breast milk. Most nickel in food remains unabsorbed in the alimentary tract and is lost in feces.

### Health Effects

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 (14 days or less), intermediate (15–364 days), and chronic (365 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 (recently reviewed in /12/. Such reactions in such persons can occur following contact with soil, bath or shower water, or metals containing nickel, as well as by handling coins or touching jewelry containing nickel. Nickel is the most frequent cause of immediate and delayed hypersensitivity, occupational as well in the general population. The metal is not only an allergen but also a potential immunomodulatory and immunotoxic agent in humans /13/.

The most serious harmful health effects from exposure to nickel, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus, have occurred in people who have breathed dust containing certain nickel compounds while working in nickel refineries or nickel-processing plants. Humans who are not sensitive to nickel must ingest very large amounts of nickel to suffer harmful health effects.

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 /14 / and the U.S. Department of Health and Human Services (DHHS /15 /). The EPA has determined that nickel refinery dust and nickel subsulfide are human carcinogens.<sup>1</sup> The concentrations of soluble and less-soluble nickel compounds in dust that were found to have produced cancers were 100,000 to 1 million times greater than the usual level of nickel in the air in the United States /2/.

No increase in the number of deaths from cardiovascular diseases has been found in workers exposed to nickel /16-18/. No studies were located

regarding ocular effects in humans or animals after inhalation exposure to nickel.

## **NICKEL TOXICITY IN PERIPHERAL TISSUES: OVERVIEW**

Many different factors have a direct impact on the toxic effects of exposure to any substance on human health. The concentration of the substance, the length of time of exposure to the substance, as well as age, gender, diet, lifestyle, health status, and family health history all play a role. About 10% of women and 2% of men in the population are highly sensitive to nickel. The type of nickel (such as metallic or oxide) affects the toxicity and the consequences for human health, as well as the ease with which it dissolves in water.

The respiratory (nickel carbonyl inhalation), oral (intake of nickel-contaminated beverages and foods), and parenteral (nickel-containing implant devices; nickel-contaminated medications) exposure routes are important for acute nickel toxicity. Inhaled nickel carbonyl is rapidly absorbed by the lungs and enters the red blood cells, where the compound undergoes conversion to  $\text{Ni}^{2+}$  and carbon monoxide. The most serious toxic effects occur when nickel is inhaled—increased risk of respiratory cancer due to chronic inhalation of fumes or fine particles when exposure is to known carcinogenic forms like nickel oxide; asthma, nasal, and sinus problems. Whereas metals like iron, copper, chromium, vanadium, cobalt undergo redox-cycling reactions, the primary route for the toxicity of mercury, cadmium, and nickel is depletion of glutathione and bonding to sulfhydryl groups of proteins /19/.

### **Acute Toxicity**

*Human studies.* Acute toxicity in humans, which results from absorption through the gastrointestinal tract or by inhalation through lungs, was first reported by Sunderman in the year 1954 /20/. The accidental inhalation of nickel carbonyl generally causes acute toxic effects in

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<sup>1</sup> <http://www.epa.gov/iris/subst/0273.htm>

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 hours to 5 days. Then delayed symptoms appear—tightness of the chest, nonproductive cough, dyspnea, cyanosis, tachycardia, palpitations, sweating, visual disturbances, vertigo, weakness, and lassitude /21/. In cases of severe nickel carbonyl poisoning, deaths have occurred 4-13 days after exposure. Acute nickel toxicity can also lead to a rise in insulin levels.

*Experimental animal models.* 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 /22/. Kidney injury and frank hematuria were also observed in acute nickel toxicity /23/. Water-soluble nickel compounds have been shown to be more acutely toxic than the less soluble ones. The single dose oral LD<sub>50</sub>s in rats for the less-soluble nickel oxide and subsulfide were > 3,600 mg Ni kg<sup>-1</sup> bwt, whereas the oral LD<sub>50</sub> for the more soluble nickel sulfate and nickel acetate ranged from 39 to 141 mg Ni kg<sup>-1</sup> bwt in rats and mice /2/.

At sublethal doses, the injection of a soluble nickel compound has been shown to impair kidney function and decrease body temperature. Sanford et al /124/ reported that an intraperitoneal injection of 3 mg Ni kg<sup>-1</sup> in rats was toxic to the kidney by inducing a decrease in Bowman's space, dilated tubules, loss of brush border, and flattened epithelia.

### Chronic Toxicity

*Human studies.* The main cause of concern when handling nickel, its alloys or its salts, is its ability to produce an allergic dermatitis. Such reactions can occur following contact with soil, bath or shower water, or metals containing nickel, as well as by handling coins or touching jewelry containing nickel. Because of its ubiquitous

occurrence in tools and articles of everyday use, nickel is the most frequent cause of immediate and delayed hypersensitivity, both occupational and among the general population (reviewed in /12/. Nickel is a premier etiologic and contributing factor of immediate antibody-mediated type allergic reactions, delayed cell-mediated type reactions, or both, from exposure through skin, mucous membranes, diet, inhalation, or nickel implants. The risk is increased risk by nickel's ready oxidation by skin exudates that promote its diffusion through the skin barrier /25/.

According to a study in humans by the EPA, /26/, nickel treatment caused irreversible lung damage, abnormal pulmonary functions, renal tubular necrosis, anemia, eosinophilia, and nasal septum ulceration. Alteration of physiological chemistry by reducing nitrogen retention, glucosurea, phosphatemia, and urinary excretion of calcium ion and zinc ion has also been observed after nickel treatment. An inhibition of ATPase activity can lead to neurological disorders, convulsions, and coma. Chronic exposure resulting in reduced nicotinamide causes a disruption in oxidative phosphorylation /27/.

*Experimental animal studies.* A chronic oral RfD of 0.02 mg kg<sup>-1</sup> d<sup>-1</sup> of nickel sulfate for two days caused a decrease in body weight and organ weight of rats /28/. Das /29/ and Dasgupta /30/ showed a nickel-induced decrease in protein synthesis in renal, hepatic, and testicular tissues of normal and low protein fed rats.

Next, following this overall perspective on the toxicology of nickel, we will discuss the effects of nickel on metabolically active peripheral tissues in general.

### NICKEL TOXICITY IN PERIPHERAL TISSUES: SPECIFIC EFFECTS

#### Hematotoxicity

*Human studies.* No human studies were located regarding hematological 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 sulfate, nickel chloride, and boric acid (estimated dose of 7.1–35.7 mg Ni/kg) /31/.

*Experimental animal studies.* A number of hematological alterations have been observed in various studies. A study in mice by Dieter et al /32/ revealed that the primary toxic effects of nickel sulfate are expressed in the myeloid system, expressed as dose-related decreases in bone marrow cellularity and in granulocyte-macrophage and pluripotent stem-cell proliferative responses. As noted by National Toxicology Program (NTP) inhalation studies in F344/N rats and B6C3F1 mice /33/, increases in hematocrit (PCV%), hemoglobin concentration, and the erythrocyte count are consistent with the production of erythropoietin in response to tissue hypoxia. Such increases could be due to nickel-induced lung damage after inhalation exposure. Another report described a decrease hemoglobin concentration in rats exposed for 6 weeks to 25 mg Ni kg<sup>-1</sup> d<sup>-1</sup> (as nickel acetate) in the diet /34/. An increase in the leukocyte count was noted in rats exposed for 28 days to 0.49 mg nickel chloride kg<sup>-1</sup> d<sup>-1</sup> in drinking water /35/. A nickel-induced increase in the platelet count in rats has been reported /36/.

Another study using nickel sulfate in rats by Das et al /37/ found that intraperitoneal administration of the compound significantly decreased the erythrocyte count, hematocrit value (PCV %), and hemoglobin 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 anemia (non-regenerative anemia) arising from injury of hematopoietic stem cells. Nickel sulfate probably

decreases all types of blood cells in rats by inhibiting bone marrow activity.

### **Immunotoxicity**

Most metals elicit a divergent immune response. Those eliciting a T-cell-mediated, delayed-type *allergic* reaction (allergic contact dermatitis) can also induce an *immunologic* antibody-mediated reaction (immunologic contact urticaria).

Because of its lack of solubility and dilution in natural deposits, no naturally occurring nickel compound (ores and minerals) is immunogenic. Nevertheless, the salts produced during the concentration process have become a potential hazard. Nickel is capable of evoking dual responses in the human immune system, sometimes in the same subject /12/. 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 /39-41/.

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 (see /12/ for review). One hypothesis is that the immunogenic activity of nickel is enhanced when Ni II is oxidized to the more reactive Ni III (or Ni IV) by endogenous reactive oxygen species (ROS) in the form of hydrogen peroxide or hypochlorite occurring in inflamed skin /42/.

*Human studies.* Human studies on the potential immunotoxicity of nickel are limited. Dental nickel has been associated with decreased numbers of T lymphocytes /13/. In cultured human cells, nickel

sulfate inhibits human keratinocyte growth and viability, augments the release of inflammatory cytokines like interleukin 1, and activates the lipoxygenase pathway in leukocytes in a dose- and time-dependent manner /43 /.

*Experimental animal studies.* One example of systemic immunotoxicity in mice is a reduction in the lymphoproliferative response to lipopolysaccharide, but this decrease was the only significant change among a panel of seven immune parameters that were evaluated /32/. In experimental animal and cell-line tests, Artik et al. /42/ found that Ni II sensitizes naïve T cells only after its bio-oxidation to Ni III or Ni IV. The ability of nickel to alter the functioning of host defense mechanisms has been demonstrated both in mice /44-45/ and in rabbits /46-47/. Experiments in rabbits revealed the ability of nickel to alter the functioning of host defense mechanisms significantly. Alveolar macrophages decreased in number and lost activity, primary antibody production was reduced, and lysozyme levels and activity significantly declined.

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. /48-49/. In a mouse model, both IgG and IgM antibody responses to KLH were significantly depressed in vivo in nickel fed animals /50/

## Liver

In both humans and animals, no effect on the hepatic system has been found after inhalation exposure. On the other hand, an oral dose of a soluble nickel compound can have profound effects on the liver.

*Human studies.* 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 sulfate /30<sup>30</sup>/.

*Experimental animal studies.* In rats, decreased liver weight was observed following exposure for 28 days to 2 years to 0.97-75 mg kg<sup>-1</sup> d<sup>-1</sup> of nickel chloride or nickel sulfate /35/. Recent studies on rats by Das et al /51/ revealed a nickel sulfate-induced degenerative effect on hepatic tissue. The group found that after the intraperitoneal injection of nickel sulfate, 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 sulfate also decreases the liver ascorbic acid and cholesterol levels in rats /51/. Das et al /52/ 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. A nickel-induced alteration of the hepatic nucleic acids concentration by decreasing DNA, RNA, and protein content in the liver has also been reported /53/. The alteration could be due to a change in gene expression by enhancing DNA methylation. Another study on rats showed swollen hepatocytes and feathery degeneration, focal necrosis, and the vacuolization of liver tissue after the animals were dermally exposed to nickel sulfate /54/.

## Lungs

*Inhalation exposure.* 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 acute respiratory distress syndrome (ARDS) occurred in a worker who sprayed a very high concentration of metallic

nickel of small particle size for 90 minutes without wearing personal protective equipment /55/. The histologic changes noted in the lungs of this case included alveolar wall damage with fibrotic changes and edema in the alveolar space. A statistically significant increase in the incidence of deaths from respiratory disease was found in welders in some studies /16/.

Human data on chronic inhalation exposure to nickel are limited to occupational exposure studies that analyzed the toxicity of nickel, usually in the form of nickel oxide, metallic nickel, or nickel refinery dust. No evidence has been found that a low-level of nickel exposure is carcinogenic in humans. Although most studies have not reported increased mortality resulting from respiratory diseases /2/, significant increases in the risk of mortality from lung or nasal cancers were found among several cohorts of nickel refinery workers. Studies of workers in other nickel industries that typically involve exposure to lower concentrations of nickel did not find significant increases in cancer risks. Although two studies also reported a higher incidence of deaths resulting from nonmalignant respiratory disease /16/, all workers in those studies were exposed to other metals (arsenic, uranium, iron, lead, chromium) as well, so whether nickel was the sole causative agent cannot be determined.

Studies in rats and mice demonstrate that chronic active inflammation in the lungs is the most prominent effect following inhalation exposure to nickel sulfate, nickel subsulfide, or nickel oxide. In acute exposure, however, chronic lung inflammation was observed at the lowest concentrations of nickel sulfate and nickel subsulfide /2/. Chronic exposure for 2 years to nickel ( $6 \text{ h d}^{-1}$ ,  $5 \text{ d wk}^{-1}$ ) resulted in active lung inflammation in mice at  $0.06 \text{ mg Ni/m}^3$  and in rats at  $0.11 \text{ mg Ni/m}^3$  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 subsulfide, and bronchiolization and/or

alveolar proteinosis in mice exposed to nickel oxide. In addition to the lung effects in rats and mice, several studies have demonstrated that exposure to nickel sulfate and nickel subsulfide can induce atrophy of the nasal olfactory epithelium /33/.

*Oral exposure.* Although no studies have been reported regarding respiratory effects in humans after oral exposure to nickel, several studies have been conducted on animal models. A significant increase in absolute and relative lung weights were observed in rats exposed for 13 weeks to  $28.8 \text{ mg Ni kg}^{-1} \text{ d}^{-1}$  as nickel sulfate in drinking water /57/. The same study also found an alteration in enzyme activities in bronchoalveolar lavage fluid and lung tissues, including an increase in protein levels and a decrease in alkaline phosphatase activity in lung tissues. Emphysema, bronchiolactasis, and cholesterol granulomas were also observed in dogs exposed to nickel (as nickel sulfate) in diet for 2 years /58/.

A recently published study by Gupta et al /59/ revealed that water-soluble nickel sulfate has profound effect on lung tissue and its antioxidant enzyme system. In this study, the intraperitoneal injection of nickel sulfate ( $2 \text{ mg/100 g bwt}$ ) 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. The study also revealed inflammation in the bronchioles, alveolar congestion, alveolar cell hyperplasia, and sometimes congestion in the lumen.

## Kidney

The most affected organ by nickel intoxication is the kidney. Different reports concern nickel exposure in humans and in experimental animal models.

*Human studies.* The man who died of ARDS after a 90-min exposure to a very high concentration of metallic nickel also had marked

tubular necrosis in the kidney /55/. Following oral exposure to nickel contaminated drinking water, a transient increase in urine albumin was reported in electroplating workers /31/. Increased levels of nickel in urine were significantly linked to urinary  $\beta_2$ -microglobulin levels in nickel refinery workers /60/. Twelve women who were occupationally exposed to soluble nickel compounds (0.75 mg Ni/m<sup>3</sup> average concentration) showed increased urinary levels of total protein,  $\beta_2$ -microglobulin, retinal binding protein, and N-acetyl- $\beta$ -D-glucosaminidase (NAG) /61/. 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 /62/.

*Animal studies.* No histologic changes have found in the kidney of rats and mice following inhalation exposure to nickel compounds. In mice exposed for 180 days to  $\geq 108$  mg Ni kg<sup>-1</sup> d<sup>-1</sup> (as nickel sulfate) in drinking water, however, the kidney was the major organ of nickel accumulation, and minor renal tubular damage at the corticomedullary junction was observed /31<sup>31</sup>/. Urinary albumin levels, a marker of glomerular barrier dysfunction, were significantly increased in nickel-exposed female rats. In rats exposed for 13 weeks to nickel sulfate in drinking water, a significant decrease in urine volume and urine glucose levels and an increase in relative kidney weight were observed /57<sup>57</sup>/.

In dogs, polyurea and increased kidney weight were found after 2 years exposure to 62.5 mg Ni kg<sup>-1</sup> d<sup>-1</sup> as nickel sulfate. In similarly treated rats, however, such increases in kidney weights were not observed /60/. Nickel induces increased kidney GPT activity, as well as decreased GOT activity in male rats, and the effects were found to be more severe in the protein-restricted dietary condition /30/. In that study, no effect on ascorbic acid-cholesterol metabolism in kidney like that found in liver was noted.

## NICKEL-INDUCED OXIDATIVE STRESS

Oxidation occurs when free radicals attack biological molecules, removing an electron. Under certain conditions, unsaturated fatty acids can undergo oxidation, known as lipid peroxidation. Lipid peroxidation sets off a chain reaction that generates large numbers of free radicals, which are both cytotoxic and genotoxic through their ability to covalently modify proteins and DNA /63-64/.

The mechanism of heavy metal toxicity through electron transfer most often involves the cross linking of the sulfhydryl groups of proteins. Free radicals can also be generated directly from molecular oxygen in a two-step process to produce superoxide anion. In the continued presence of heavy metals, the superoxide anions formed can then combine with protons in the dismutation reaction, generating hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the process. Superoxide anions can also produce highly toxic hydroxyl radicals.

Suitable mechanisms have evolved so that the steady-state concentrations of potentially toxic oxygen-derived free radicals are kept in check under normal physiological conditions by the body's intrinsic antioxidant defense system. Nevertheless, the enhanced generation of these ROS can overwhelm the intrinsic defenses of the cell, resulting in a condition known as *oxidative stress*.

### Ni(II) Induces Oxidative Stress

Ni(II) enhances the oxidation of all DNA bases in vitro (reviewed in /65/ and lipid peroxidation in vivo /66-67/. Nickel-induced oxidative stress is rather weak, however, depleting glutathione and oxidatively activating various transcription factors /67-70<sup>67</sup>/. Although nickel(II) by itself does not cause efficient free radical generation from oxygen, H<sub>2</sub>O<sub>2</sub>, or lipid hydroperoxides, the reactivity of Ni(II) with those oxygen derivatives can be modulated by chelation with certain histidine- and cysteine-containing ligands /71-72/.

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 /73-74/. 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.

### Human Studies

The oxidative effects of nickel on human lymphocytes in vitro, manifested as increased levels of intracellular ROS, lipid peroxidation, and hydroxyl radicals after acute exposure to inorganic nickel, support the concept of nickel chloride-induced oxidative stress /74/. In the human bronchial epithelial cell line BEAS-2B, however, nickel was only mildly active in inducing an oxidative stress response as compared with other metal species as measured by ROS induction /75/. Arranging various metal species in order of increasing toxicity yields Ni(II) << Cr(VI) << Cd(II).

The results of a series of studies using cultured human peripheral blood lymphocytes also suggest that nickel induces oxidative stress in humans /76-80/. In an in vitro study using human lymphocytes, nickel sulfate increased the generation of hydrogen peroxide and lipid peroxidation /76/. The participation of oxidative stress involving H<sub>2</sub>O<sub>2</sub>, the superoxide anion radical, the hydroxyl radical, and iron was also shown in nickel carbonate hydroxide-induced genotoxic responses and lymphocyte death /77/. The pretreatment of human blood lymphocytes with either CAT (a H<sub>2</sub>O<sub>2</sub> scavenger), or SOD (a scavenger of O<sub>2</sub><sup>-</sup> radical) significantly reduced markers of nickel carbonate hydroxide-induced genetic and cellular damage.

Glutathione depletion, a marker of oxidative stress, was found in a human alveolar epithelial

Type II-like cell line after treatment with nickel-containing ultrafine metal dusts /81/. 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 /82/.

After controlling for confounders, plasma lipid peroxidation levels were significantly increased and erythrocyte antioxidants were significantly decreased in a group of nickel-plating workers and their helpers as compared with controls /83/.

### Experimental Animal Studies

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 /84/. The level of MDA was also found to be significantly elevated in serum of nickel chloride-treated rats /85/. Our group /52/ found that nickel sulfate treatment significantly increases the production of hepatic lipid peroxides in rats.

Using a mouse model, Anderson and Anderson /86/ showed that intraperitoneal administration of nickel chloride enhances hepatic lipid peroxidation and depletes glutathione. In male ICR mice, both hepatic lipid peroxidation and nickel levels were enhanced after a single intraperitoneal injection of nickel chloride /87/. In another mouse study, multiple intraperitoneal doses of the compound elicited a moderate increase in lipid peroxidation in whole testis homogenates and higher dose-related increases in both mitochondrial and microsomal fractions /88/.

Misra and coworkers /89/ showed that a single intraperitoneal injection of nickel (II) acetate increases lipid peroxidation and glutathione-S-transferase activity in rat liver and kidney while concomitantly decreasing the glutathione concentration and glutathione reductase activity. 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 /89-90/. The hepatic glutathione levels in all strains were inhibited by nickel in a strain-dependent manner, in the following order of inhibition: C57BL/6NCr mice > BALB/cAnNCr  $\geq$  B6C3F1 > C3H/HeNCr-MTV /90/. 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 /91/. Another in vitro study showed that the exposure of rat lymphocytes to nickel subsulfide increases the formation of ROS in a concentration-dependent manner /81/.

#### ANTIOXIDANTS: ROLE OF ASCORBIC ACID

Because of the oxygen-rich environment in which proteins exist, reactions with ROS are unavoidable. Experimental data suggest that antioxidants could play an important role in protecting proteins against oxidative damage caused by free radicals and in abating the genotoxic hazards of nickel in vitro /92/. Antioxidants are intimately involved in the prevention of cellular damage by interacting with free radicals and by terminating the chain reaction, thereby curtailing free radical activity.

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 /93/.

Numerous reports have shown the positive effect of vitamin C as an antioxidant and scavenger of free radicals. On the other hand, doses of Vitamin C exceeding the daily recommended dietary allowance could lead to the formation of toxic advanced glycation endproducts, which are derived from the degradation products of carbohydrates, lipids, and

ascorbic acid /94-96/. Advanced glycation endproducts are a major contributing factor to diabetic complications and aging. Such adverse effects of vitamin C could be associated only with supplemental intake, however.

#### Nickel-Induced Depletion of Ascorbic Acid

Nickel compounds adversely affect cells by modulating ascorbic-acid metabolism and other metabolic pathways /29, 51/. One in vitro study using a human airway epithelial cell line found that the depletion of intracellular ascorbate by nickel results in the induction of hypoxic stress /97/. The authors 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-1 $\alpha$  dependent gene expression.

#### Human Studies

Recent reviews of human studies have reported either a vitamin C-mediated reduction in oxidative DNA damage or a null effect /98, 99/. In patients with systemic lupus erythematosus, the combined administration of vitamins C and E was associated with a significant decrease in plasma MDA, a reactive product of lipid peroxidation /100/. A recent demonstration that Vitamin C conjugates lipid peroxidation products in the plasma of healthy human subjects elicited the hypothesis that this reaction could be a biologically relevant pathway for the elimination of electrophilic lipid peroxidation products /101/.

On the other hand, data from a controlled study in humans showed no evidence of either a protective or deleterious effect of a single dose of Vitamin C and/or Vitamin E on DNA damage, resistance of DNA to oxidant challenge, or lipid peroxidation /102/. In another randomized,

placebo-controlled, double blind, 2-way cross over trial in healthy males, vitamin C did not alter either the formation or the breakdown of plasma MDA /103/.

### Experimental Animal Models

Numerous animal studies support the ability of vitamin C to protect against free radical damage in vitro. 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 /51, 104/. Increased lipid peroxidation could stimulate phospholipase A<sub>2</sub> (PLA<sub>2</sub>) 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 /105/, leading to functional impairment and tissue damage. The administration of L-ascorbic acid can improve the situation in rats by its scavenging activity within the lipid region of the membrane /104/. Moreover, ascorbic acid protects glutathione from nickel abuse and maintains cellular thiol status in liver. Treatment with ascorbic acid significantly lowered the levels of lipid peroxidation in nickel-treated rats /85/. Ascorbate has also been reported to prevent nickel induced alteration of the serum lipid profile in rats by lowering serum total cholesterol, low density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) levels along with increasing the level of serum high density lipoprotein cholesterol (HDL-C) /36, 51/. Ascorbic acid counteracts the nickel-induced changes in 3-hydroxy 3-methyl glutaryl coenzyme A (HMG-CoA) reductase activity, thereby improving the serum lipid profile and lowering the total cholesterol level /106/. The oral administration L-ascorbic acid simultaneously with nickel sulfate treatment increases the DNA, RNA,

and protein content of hepatic cells to a great extent /52/. Another experimental study revealed that ascorbate has a potent role in the antioxidant defense system in erythrocytes /35/. Nickel induced a loss of membrane function by elevating the levels of the erythrocyte malondialdehyde (MDA) level, a measure of oxidative stress, as well as by altering the GSH level or antioxidant enzymes like SOD, CAT, and GSH-Px activities. The simultaneous treatment with ascorbic acid, however, protected from this toxic effect of nickel.

In nickel-treated rats, L-ascorbate supplementation leads to an overall improvement of the antioxidant defense system in the lungs of the experimental animals /59/. A histopathologic study showed that the adverse change in hepatocellular integrity following nickel sulfate administration was improved by the simultaneous administration of L-ascorbic acid /36/. Nickel sulfate also resulted in an inflammation of the bronchioles, alveolar congestion, and alveolar cell hyperplasia, but simultaneous treatment with L-ascorbate induced a remarkable improvement of the histopathological architecture of lung tissues to a near-normal condition /59/.

### CONCLUSION

We can conclude from the various studies cited, including those conducted by the present author and his group, that nickel is a potent hematotoxic, hepatotoxic, pulmonary toxic, and nephrotoxic agent. Although conflicting results have been reported in human studies of the effect of vitamin C supplementation on oxidative stress, the experimental animal results with ascorbic acid suggest that a high consumption of dietary vitamin C might ameliorate nickel-induced free-radical disease.

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