CHAPTER I

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Nickel

General description

Nickel (Ni) is a silvery-white, hard metal. Although it forms compounds in several oxidation states, the divalent ion seems to be the most important for both organic and inorganic substances, but the trivalent form may be generated by redox reactions in the cell (Huang et al., 1993). Nickel compounds that are practically insoluble in water include carbonate, sulfides (the main forms being amorphous or crystalline monosulfide, NiS, and subsulfide Ni$_3$S$_2$) and oxides (NiO, Ni$_2$O$_3$). Water-insoluble nickel compounds may also dissolve in biological fluids (Yamada et al., 1993). Particles of the same chemical entity (oxides and sulfides) have different biological activity depending on crystalline structure and surface properties (Geets et al., 2006). Soluble nickel salts include chloride, sulfate and nitrate. In biological systems, nickel forms complexes with adenosine triphosphate, amino acids, peptides, proteins and deoxyribonucleic acid.

Sources

Nickel is widely distributed in nature, forming about 0.008% of the earth's crust. The core of the earth contains 8.5% nickel, deep-sea nodules 1.5%; meteorites have been found to contain 5–50% nickel (IARC, 1990). The natural background levels of nickel in water are relatively low, in open ocean water 0.228–0.693 µg/litre, in fresh water systems generally less than 2 µg/litre. Agricultural soils contain nickel at levels of 3–1000 mg/kg (WHO, 1991). The nickel content is enriched in coal and crude oil. Nickel in coals ranges up to 300 mg/kg; most samples contain less than 100 mg/kg but there is a large variation by region. The nickel content of crude oils is in the range <1–80 mg/kg (WHO, 1991).

Production and use

There are two commercial classes of nickel ore, the sulfide ores (pentlandite and pyrrhotite) and the silicate-oxide. Intermediate uses of nickel include 42% in steel production and 36% in the production of other alloys. Electroplating in the form of nickel sulfate accounts for about 18%. The most important end users are
transportation 23%, chemical industry 15%, electrical equipment 12%, and construction 10% (WHO, 1991). Nickel in coinage, other manufactured products and household appliances may be important for some health effects (dermatitis). The burning of residual and fuel oils, nickel mining and refining, and municipal waste incineration are the main anthropogenic sources of nickel emissions to the atmosphere (WHO, 1991). These sources account for about 90% of the total global emission, estimated to be $42.85 \times 10^6$ kg/year. The major nickel species in ambient air is nickel sulfate. This soluble ("leachable") form is estimated to comprise 60–100% (Profumo et al., 2003), or 15–93% (average 54%). Estimates of emission from natural sources vary in the range $8.5–160 \times 10^6$ kg/year (WHO, 1991).

**Occurrence in air**

Because of the large number of nickel-releasing sources, the nickel concentration in ambient air may show considerable variation. In a remote area (Canadian Arctic) levels of 0.38–0.62 ng/m$^3$ were recorded (Hoff and Barrie, 1986), as compared to 124 ng/m$^3$ in the vicinity of a nickel smelter (Chan and Lusis, 1986) with highest value recorded was 64 ng/m$^3$ (Norseth, 1994). Pentlandite $[(\text{FeNi})_9\text{S}_8]$ and nickel in the silicate zone (also called the garnierite zone) are two naturally occurring forms of nickel found in rocks. Nickel from man-made sources is probably represented mostly by oxides and sulfates of rather small particle size (mass median diameter (MMD) about 1 $\mu$m) and some 15–90% is soluble (leachable). Occupational studies of nickel exposure have not provided dose-specific estimates of risk for individual species, and only rarely total exposure estimates that are comparable between the different plants. The MMD of nickel in urban air is 0.83–1.67 $\mu$m, and less than 1 $\mu$m in 28 55% of particles (Goforth and Christoforou, 2006).

**Analytical methods**

Absorption on cellulose ester membrane filters followed by wet digestion and analysis by electrothermal atomic absorption spectrometry (ET-AAS), inductively coupled plasma atomic emission (ICP-AES) or ICP-mass spectrometry are suitable for analysis of nickel in air, with a detection limit of 5 ng/sample (Templeton et al., 1994b). ET-AAS with Zeeman background correction is currently the most common technique for determining nickel in biological
materials. Detection limits of 0.4 mg/litre for urine and 0.05µg/litre for serum have been reported. It is important to exclude sample contact with nickel-containing materials (e.g. steel syringes) (Templeton et al., 1994a; Sunderman, 1993a).

**Routes of exposure**

The main routes of nickel intake for humans are inhalation, ingestion and absorption through the skin.

**Air**

Assuming a daily respiratory rate of 20 m³, the amount of airborne nickel entering the respiratory tract is in the range 0.1–0.8 µg/day when concentrations are 5–40 ng/m³ in ambient air. Owing to the variation in particle size and solubility between nickel compounds, no general statements can be made on the retention or absorption of nickel in the respiratory tract (Oberdörster, 1992). A total deposition of about 50% of the inhaled dose was estimated for particles with an MMD of 2.0 µm, while deposition was about 10% for those of 0.5 µm. For larger particles, more than 50% of the deposited dose was in the nasopharyngeal part of the respiratory tract as against less than 10% for the smaller particles.

In a single experiment, 95% of the nickel in a respirable aerosol of nickel-enriched fly ash was retained in the lung one month after the exposure (Di Pietro et al., 2009). Following intratracheal administration of nickel chloride, only 0.1% was retained in the lungs of rats at day 21 (Costa et al., 2006). About 0.04–0.58 µg of nickel is released with the mainstream smoke of one cigarette (WHO, 1991). Smoking 40 cigarettes per day may thus lead to inhalation of 2–23 µg of nickel. The possibility that nickel occurs in mainstream smoke in part as nickel carbonyl has never been substantiated.

**Drinking-water**

Nickel concentrations in drinking-water in European countries of 2–13 µg/litre have been reported (Alam et al., 2008). An average value of 9µg/litre and a maximum of 34µg/litre were recorded in Germany (Schulz et al., 2007). Nickel may, however, be leached from nickel-containing plumbing fittings, and levels of up to 500 µg/litre have been recorded in water left overnight in such fittings (Andersen, 1983). In areas with nickel mining, levels of up to 200µg/litre have been
recorded in drinking-water. The average level of nickel in drinking-water in public water supply systems in the United States was 4.8μg/litre in 1969. Assuming a concentration of 5–10 μg/litre, a daily consumption of 2 litres of drinking-water would result in a daily nickel intake of 10–20 μg.

**Food**

In most food products, the nickel content is less than 0.5 mg/kg fresh weight. Cacao products and nuts may, however, contain as much as 10 and 3 mg/kg, respectively. Total diet studies indicate a total average oral intake of 200–300 μg/day (WHO, 1991). Recovery studies indicate an absorption rate of less than 15% from the gastrointestinal tract (Sunderman et al., 1988b).

**Relative significance of different routes of exposure**

Percutaneous absorption of nickel is quantitatively minor, but is the most significant for cutaneous manifestations of nickel hypersensitivity (Theler et al., 2009). Iatrogenic exposure to nickel may occur as a result of dialysis treatment, prostheses and implants, and medication. Such exposure is of minor importance for practical purposes. Ear-piercing, however, increases the probability of nickel sensitization (Nielsen and Menne, 1993). Table 1 summarizes the levels of daily nickel intake by humans from different routes of exposure.

<table>
<thead>
<tr>
<th>No.</th>
<th>Type/route of exposure</th>
<th>Daily nickel intake</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Foodstuffs</td>
<td>&lt;300</td>
<td>45 (&lt;15%)</td>
</tr>
<tr>
<td>2</td>
<td>Drinking-water</td>
<td>&lt;20</td>
<td>3 (&lt;15%)</td>
</tr>
<tr>
<td>3</td>
<td>Ambient air (urban dweller)</td>
<td>&lt;0.8</td>
<td>0.4 (50%)</td>
</tr>
<tr>
<td>4</td>
<td>Ambient air (smoker)</td>
<td>&lt;23</td>
<td>12 (50%)</td>
</tr>
</tbody>
</table>

Both the gastrointestinal and respiratory uptake rates have been estimated on the basis of very limited experimental evidence. Gastrointestinal uptake is of limited interest for effects other than nickel hypersensitivity. Moreover, even
though a low-nickel diet has been reported to improve clinical symptoms in some hypersensitive individuals, other factors seem to be more important. As the respiratory tract is a major target organ as well as an uptake organ for nickel, inhalation is the most significant route of exposure with regards to lung effects. Retention in the respiratory tract is more important than uptake into the general circulation because respiratory cancer is the critical effect. Given the particle distribution in ambient air, an approximate 50% retention figure seems reasonable for risk estimation. Effects in the lung resulting from oral intake cannot be excluded. Inhibition of 5′-nucleotidase activity and enhanced lipid peroxidation in pulmonary alveolar macrophages has been demonstrated in the respiratory tract following parenteral injection of nickel chloride in rats (Sunderman et al., 1988c). The relative importance for tumour development of respiratory tract exposure from the general circulation is not known.

**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 (Proietti et al. 2005). A fatal case of nickel poisoning was reported for a 2 ½ yr old girl who had ingested 15 g of nickel sulphate (Daldrup et al., 1983). 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 (Rendall et al., 1994). 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) (Gurjar and Mohan, 2003). 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 (Horvath et al., 1995). Kidney injury and frank haematuria were also observed in acute nickel toxicity. 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 $> 3600$ 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 (ATSDR, 2005).

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/m$^3$) caused an increase in airway and eye irritations, headaches, and tiredness (Luo et al. 2009). Bwititi and Ashorobi (1998) 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 $\mu$g /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. Toxic symptoms like lethargy, ataxia, hypothermia, salivation, diarrhoea were observed in the nickel treated rats at 10mg/kg/d doses30. The mortality rates among rats are very high in dose-dependent nickel treatment (IRIS, 1996). Obone et al (1999) 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 223.5mg/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 12.5mg/kg and 25mg/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 (Obone et al., 1999). 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, $\alpha_2$-microglobulin, retinal binding protein, and N-acetyl-$\alpha$-D- glucosaminidase (NAG) (Vyskocil et al., 1994). Although altered biomarkers reflected tubular dysfunction, no effect was found on markers of glomerular function, urinary albumin levels, or transferrin levels.

Earlier studies from our laboratory have reported hepato and renal toxicity of nickel salts (Parekh and Rao, 2003). Metabolic and histomorphological changes
have been elaborated by in 30 days of nickel chloride administration in swiss albino male mice. Reproductive toxicity by nickel administration has been illustrated by Seema et al. (2003) in ovary of mouse.

**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 (USAF, 1990). 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 (NAS, 1975). A longtime exposure of rats to nickel oxide (42mg nickel/m³) produced emphysema and other proliferative and inflammatory changes (Oller et al., 2008). It was reported by Jadhav al (2007) that drinking water contains NiSO₄ 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. Chashchin et al., (1994) reported on possible reproductive and developmental effects in humans of occupational exposure to nickel (0.13-0.2 mg nickel/m³). 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 (NTP, 1996a).

**Population groups at high probability of exposure**

Industrial activity accounts for most of the variability of nickel deposition on the earth's surface, but deposits from meteorites and volcanic eruptions may
exceed releases from anthropogenic sources (Brimblecombe, 1994). Point-source emission increases nickel exposure, but an impact on health from such emissions has not been convincingly documented (Norseth, 1994).

Little is known about risk groups in the general population, although smokers and those exposed at work have higher exposures than other groups within the population. Nickel concentrations in workroom air, particularly in the refining industry may be significantly increased compared to those in ambient air. An increased cancer risk has been repeatedly demonstrated in the refining industry, but not for secondary users of nickel. Workroom air levels of nickel in secondary and end-users of nickel are generally much lower than in the refining industry, often by a factor of 10–100 (ICNC, 1990). Exposure levels in workroom air in the refining industry have been estimated at 1–5 mg/m\(^3\) for soluble nickel and from less than 2mg /m\(^3\) to more than 9mg /m\(^3\) for sulfidic nickel. Exposure to oxidic nickel may have exceeded 10 mg/m\(^3\). In addition, mixed exposures have been the rule rather than the exception. Secondary users of nickel are usually exposed to less than 0.1 mg/m\(^3\) with occasional levels of up to 1 mg/m\(^3\) (IARC, 1990, ICNC, 1990).

**Toxicokinetics**

**Absorption**

At least 50% of a single inhaled dose of nickel carbonyl is absorbed, the agent passing the alveolar wall intact (WHO, 1991). Few data exist on the absorption of nickel from particulate matter deposited in the respiratory tract. The upper limit for particle retention may be calculated from respiratory deposition and retention models, but such calculations are of limited practical value because of the different biological availability of nickel compounds. Absorption of nickel into the blood may be of limited significance as particles retained in the cells of the respiratory tract are more important. Soluble nickel compounds are rapidly removed from the lung. For example, Cempel and Janicka, (2002) demonstrated that only 0.1% of the dose was found in the lungs 21 days after tracheal instillation of nickel chloride in rats. A steady-state lung burden was observed at a concentration of 90\(\mu\)g/m\(^3\), as predicted from computer modelling, while the lung burden continued to increase with repeated exposure to 400\(\mu\)g/m\(^3\). In syrian hamsters exposed to artificial nickel oxide aerosols (unspecified; MMD 1.0–2.5\(\mu\)m), 20% of inhaled
nickel remained after the initial elimination, and 45% of this was still present after 45 days (Kerckaert et al., 1996). Oberdörster has considered lung dosimetry at length, using animal–human extrapolation modeling (Oberdörster, 1992). Equivalent human exposure concentrations were calculated on the basis of results in rats (Oberdörster, 1992). The model depends heavily on particle size and solubility; further knowledge of the kinetics of inhaled nickel compounds and on mechanisms of clearance and tumorigenicity is needed for reliable modelling and risk estimation.

Distribution

The main carrier protein of nickel in serum is albumin, but nickel is also bound to β-2 macroglobulin and histidine (Sunderman, 1993b). The body burden of nickel in adult humans averages about 0.5 mg per 70 kg. The highest concentrations of nickel are found in the lung and in the thyroid and adrenal glands (about 20-25 μg/kg wet weight). Most other organs (e.g. kidney, liver, brain) contain about 8-10 μg/kg wet weight (Torres and Johnson, 2001). Following parenteral administration to experimental animals, the kidney invariably showed the highest concentrations of nickel followed by either the lung or the pituitary glands (Bwititi et al., 1998). Reference values for nickel concentrations in serum and urine from healthy persons without occupational exposure to nickel compounds have been compiled (Templeton et al., 1994a; Sunderman, 1993a). Values for serum/plasma are in the range 0.14-0.65 μg/litre; values of around 0.2μg/litre seem to be the most reliable. Corresponding values for urine are 0.9-4.1 μg/litre, with values of 1-2 μg/litre the most reliable. For whole blood, values of 0.34-1.4 μg/litre are given. These values are substantially lower than those reported prior to 1980 because of better analytical methods and improved control of contamination. The metal concentrations in the different samples were not influenced by age or sex. Various diseases (myocardial infarction, acute stroke, thermal burns and hepatic cirrhosis) influence the kinetics of nickel metabolism.

Metabolism and elimination

Nickel may undergo redox metabolism generating the trivalent form thus forming reactive oxygen species. The intracellular release of nickel ion following phagocytosis of particles of oxidic and/or sulfidic nickel is an important metabolic
pathway. Minute particles containing nickel have been demonstrated close to the nuclear membrane. Nickel ions may also enter the cell directly. Parenteral administration of nickel induces changes in the tissue distribution of other metals, and several physiological divalent cations influence nickel metabolism. Specifically, manganese inhibits the dissolution of nickel subsulfide in rat serum, and inhibits phagocytosis of nickel subsulfide particles (IARC, 1990). Unabsorbed nickel in the gastrointestinal tract is lost in the faeces (reflecting the daily dietary intake). Figures of 180–250 µg/day should be expected on the basis of an estimated daily intake of 200-300 µg and absorption of less than 15%. Excretion of 258µg/day has been reported (Sunderman, 1993a).

Absorbed nickel is eliminated in the urine. Excretion via sweat, secretion via saliva and deposition in hair has been reported. However, urinary excretion is the main clearance route. The biological half-time of nickel depends on the nickel species tested. For soluble compounds, the half-time of plasma nickel is 11–39 hours in humans; for particulate compounds, half-times of 30–54 hours have been recorded (ATSDR, 2005). A urinary elimination half-time of 17–48 hours has been reported for the absorbed dose following accidental exposure in humans (Schaller et al., 2007). Protracted retention and gradual elimination from body pools (respiratory organs) take place following exposure to nickel particulates in welding fumes (Schaller et al., 2007).

**Biomarkers of exposure**

Both plasma and urine concentrations of nickel are useful biomarkers of nickel inhalation exposure on a group basis (Mari et al., 2009). The correlation between exposure and biological values on an individual basis is low and significant only in some investigations involving exposure to soluble compounds. The levels in plasma and urine are highly dependent on the nickel species in air. High air levels of oxidic and sulfidic nickel give relatively lower plasma and urine values than a corresponding level of soluble chlorides or sulfates, but higher values in the nasal mucosa and probably also in the lungs (possible target organs) (Sunderman, 1993b).
Health effects

There is evidence that nickel is an essential trace element in several animal species, plants and prokaryotic organisms. Nickel appears to be essential for humans, although no data are available concerning nickel deficiency.

Effects on experimental animals and in vitro test systems

Toxicological effects

Inhalation of all types of nickel compounds induces respiratory tract irritation, chemical pneumonia, emphysema and varying degrees of hyperplasia of pulmonary cells, and fibrosis (pneumoconiosis) (IARC, 1990). Nickel may precipitate autoimmune phenomena and induce immunosuppression in vitro; the clinical importance of such effects has not been reported (Pelletier et al., 1990). Nickel can cross the placental barrier, thus being able to influence prenatal development by direct action on the embryo. Fetal death and malformations have been reported following injection of various species of nickel compounds in experimental animals (IARC, 1990).

Mutagenic effects and effects identified by other in vitro assays

Negative mutagenicity data were obtained in most bacterial test systems owing to lack of absorption, but many nickel compounds can induce in vitro mammalian cell transformation and are clastogenic to various degrees (IARC, 1990).

Critical organs, tissues and effects

The critical organ following inhalation exposure is the respiratory tract. After short-term high-dose inhalation exposure, lung irritation and pneumonia are critical effects. Increased mortality of nonmalignant respiratory disease has been reported in nickel refinery workers with more than 5 years of exposure, and pneumoconiosis has been reported following 12–20 years of exposure. No details on nickel compounds or exposure levels was given, but nickel oxide (Ni₂O₃) was found to be fibrogenic when instilled intratracheally (WHO, 1991). Tumour induction must, however, be regarded as the critical effect. Ingestion of high doses of nickel salts causes gastric irritation and the skin can be considered as a target
organ with dermatitis as a critical effect (WHO, 1991). The lung is the critical organ following nickel carbonyl inhalation, the effect being pulmonary oedema.

Severe lung damage has been recorded following acute inhalation exposure to nickel carbonyl. Reversible renal effects (in workers), allergic dermatitis (most prevalent in women), and mucosal irritation and asthma (in workers) have been reported following exposure to inorganic nickel compounds (IARC, 1990). Renal effects and dermatitis presumably relate both to nickel uptake by both inhalation and ingestion, in addition to cutaneous contact for dermatitis. Allergic skin reactions to nickel (dermatitis) have been documented both in nickel workers and in the general population. However, the significance of nickel as a cause of occupationally-induced skin reaction is decreasing. In contrast, there is evidence that nickel is increasingly a major allergen in the general population, especially in women. About 2% of males and 11% of females show a positive skin reaction to patch testing with nickel sulfate. Ear-piercing considerably increases the risk of nickel sensitization (Nielsen and Menne, 1993). The respiratory tract is also a target organ for allergic manifestations of nickel exposure. Allergic asthma has been reported among workers in the plating industry following exposure to nickel sulfate. Cytogenic studies have been conducted in workers in the nickel-refining industry (crushing, roasting, smelting and electrolysis), in nickel carbonyl production, and in electroplating. Elevated levels of sister chromatid exchanges and chromosomal aberrations have been demonstrated in workers in nickel refining plants and in nickel platers; the main effect was chromosomal gaps (IARC, 1990). No effects were found in workers exposed to nickel carbonyl (IARC, 1990). Boysen et al., (1982) suggested the use of histopathological changes in the nasal mucosa as a biomarker of effect in refinery workers and later concluded that such results at best could indicate groups of persons at increased risk for nasal carcinoma.

Parekh et al., (2003) reported severe damage in histoarchitecture and ultrastructural changes in liver and kidney of mice treated with nickel chloride at doses of 8mg/kg and 16mg/kg. The results accompanied with metabolic impairment and increased ROS. Combination of nickel and chromium showed synergetic toxic effect on vital tissues of mice than compared single toxicant. Ovarian cyclicity was significantly altered along with increased oxidative stress in the ovaries of female mice treated with nickel for 30 days (Seema et al., 2003).
Carcinogenic effects

Studies linking nickel uptake from the environment and cancer incidence in the general population are not available. There is agreement that nickel refinery workers exposed by inhalation to various nickel compounds in the past are at a significantly higher risk for cancer of the lungs and the nasal cavity than the non-occupationally exposed population (IARC, 1990). Laryngeal cancer, kidney cancer, and cancer of the prostate or bone have also been found in nickel workers, but the epidemiological evidence does not indicate a relationship to nickel exposure or to any other occupational origin (ICNC, 1990). At the Clydach refinery, Wales, a high relative risk of nasal and lung cancer has been associated with inhalation exposure in the calcining, roasting and leaching departments before 1920. Much of the risk was related to work at the linear calciner where nickel exposure levels were 10–100 mg/m3 with a composition of about 60% oxidic, 20% sulfidic, 20% metallic and 3% soluble nickel. Even if the exposure to soluble nickel compounds is low compared to that to the particulate form, analysis indicates that exposure to soluble forms together with the oxidic or sulfidic forms increases the risk. The decrease in nickel air concentrations to a maximum of 20 mg/m3 in the workplace after 1930 seems to explain the decrease in risk, even if other changes (lower copper and sulfur in the feed) in the production technology also took place (ICNC, 1990). Very high relative risks of nasal and lung cancer have also existed in the calcining, roasting and leaching departments of refineries in Canada (INCO, Ontario) and Norway, (Falconbridge, Kristiansand). Exposure levels in the same range as in Clydach were recorded in Canada, somewhat lower (>10 mg/m3) in Norway (ICNC, 1990). In Norway, oxidic nickel was reduced to concentrations of less than 5 mg/m3 after 1955, but an increased risk of lung cancer was still recorded in a cohort with first job entry after 1956 (Andersen, 1992). In a recent follow-up, the highest risk was found among those with the highest estimated dose of soluble nickel. There appeared to be a multiplicative effect for smoking and exposure to total nickel (Andersen et al., 1996). Studies of industrial secondary and end-users of nickel have generally not shown carcinogenic effects, but the exposure levels have been less than 1 mg/m3 both for particulate and for soluble nickel compounds.
Ni(II) induced 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 (Valko et al., 2006). 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 (Lynn et al., 1998). 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 (Ni²⁺) 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. 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 hypoxia-inducible factor HIF-1-dependent genes (Salnikow et al., 2000). Ni (II) enhances the oxidation of all DNA bases in vitro (Salnikow et al., 1994) and lipid peroxidation in vivo (Coogan et al., 1989). Although nickel(II) by itself does not cause efficient free radical generation from oxygen, H₂O₂, or lipid hydroperoxides, the reactivity of Ni(II) with those oxygen derivatives can be modulated by chelation with certain histidine- and cysteine-containing ligands (Shi et al., 1993; Shi et al., 1992). 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 (Chen et al., 2003a). 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 (Das and
Buchner, 2007). The results of a series of studies using cultured human peripheral blood lymphocytes also suggest that nickel induces oxidative stress in humans (M'Bemba-Meka et al., 2007; M'Bemba-Meka et al., 2005). 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 (M’Bemba-Meka et al., 2005).

The pretreatment of human blood lymphocytes with either CAT (a H$_2$O$_2$ 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 (Wozniak and Blasiak, 2002). 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 (Chen et al., 2003b). 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 (Sunderman et al., 1985). The level of MDA was also found to be significantly elevated in serum of nickel chloride-treated rats (Sunderman et al., 1988c). 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 (Misra et al., 1990). 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 (Misra et al., 1990; Rodriguez et al., 1991). 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 (Chakrabarti and Bai, 1999).

Evaluation of human health risks

Exposure evaluation

Nickel is present throughout nature and is released into air and water both from natural sources and as a result of human activity. In nonsmokers, about 99% of the estimated daily nickel absorption stems from food and water; for smokers the figure is about 75%. Nickel levels in the ambient air are in the range 1–10 ng/m$^3$ in urban areas, although much higher levels (110–180 ng/m$^3$) have been recorded in heavily industrialized areas and larger cities. There is, however, limited information on the species of nickel in ambient air. Consumer products made from nickel alloys and nickel-plated items lead to cutaneous contact exposure. Exposure to nickel levels of 10–100 mg/m$^3$ have been recorded for occupational groups, with documented increased cancer risk. Exposure levels in the refining industry are currently usually less than 1–2 mg/m$^3$, often less than 0.5 mg/m$^3$. Experimental and epidemiological data indicate that the nickel species in question is important for risk estimation.

Health risk evaluation

Allergic skin reactions are the most common health effect of nickel, affecting about 2% of the male and 11% of the female population. Nickel content in consumer products and possibly in food and water are critical for the dermatological effect. The respiratory tract is also a target organ for allergic manifestations of occupational nickel exposure. Work-related exposure in the nickel-refining industry has been documented to cause an increased risk of lung and nasal cancers. Inhalation of a mixture of oxidic, sulfidic and soluble nickel compounds at concentrations higher than 0.5 mg/m$^3$, often considerably higher, for many years has been reported (ICNC, 1990). Nickel has a strong and prevalent
allergenic potency. In addition, nickel and an alloy with 66.5% nickel and 12.5% chromium caused tumours following tracheal instillation. A corresponding instillation with an alloy of 26.8% nickel and 16.2% chromium had no such effect, indicating that it was nickel and not chromium which caused the tumours. The tumorigenic potency varies with chemical composition, solubility and particle surface properties (UEPA, 1986).

On the basis of the underlying concept that all nickel compounds can generate nickel ions which are transported to critical sites in target cells, IARC classified nickel compounds as carcinogenic to humans (group 1) and metallic nickel as possibly carcinogenic to humans (group 2B) (IARC, 1990). WHO estimated an incremental unit risk of $4 \times 10^{-4} (\mu g/m^3)^{-1}$ calculated from epidemiological results (WHO, 1987). On the basis of epidemiological studies, EPA classified nickel dust as a class A carcinogen and estimated the lifetime cancer risk from exposure to nickel dust to be $2.4 \times 10^{-4}$. This estimate placed nickel in the third quartile of the 55 substances evaluated by the EPA Carcinogen Assessment Group with regard to their relative carcinogenic potency (UEPA, 1986). Assuming a content of 50% of nickel in total dust, a unit risk of $4.8 \times 10^{-4}$ was estimated for this compound. An estimate of unit risk can be given on the basis of the report of lung cancer in workers with first employment in 1968–1972 followed through to 1987 in Norway (Andersen et al., 1996). Using the estimated risk of 1.9 for this group and an exposure of 2.5 mg/m$^3$, a lifetime exposure of 155 $\mu g/m^3$ and a unit risk of $3.8 \times 10^{-4} (\mu g/m^3)^{-1}$ can be calculated.

**Guidelines**

Even if the dermatological effects of nickel are the most common, such effects are not considered to be critically linked to ambient air levels. Nickel compounds are human carcinogens by inhalation exposure. The present data are derived from studies in occupationally exposed human populations. Assuming a linear dose–response, no safe level for nickel compounds can be recommended. On the basis of the most recent information of exposure and risk estimated in industrial populations, an incremental risk of $3.8 \times 10^{-4}$ can be given for a concentration of nickel in air of 1 $\mu g/m^3$. The concentrations corresponding to an excess lifetime risk of 1:10 000, 1:100 000 and 1: 1 000 000 are about 250, 25 and 2.5$\mu g/m^3$, respectively.
Curcumin

Turmeric was described as *C. longa* by Linnaeus and its taxonomic position is as follows:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae, Plants</th>
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<tbody>
<tr>
<td>Division</td>
<td>Angiosperma</td>
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<tr>
<td>Class</td>
<td>Liliopsida</td>
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<tr>
<td>Subclass</td>
<td>Commelinids</td>
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<tr>
<td>Order</td>
<td>Zingiberales</td>
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<tr>
<td>Family</td>
<td>Zingiberaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Curcuma</td>
</tr>
<tr>
<td>Species</td>
<td><em>Curcuma longa</em></td>
</tr>
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</table>

The wild turmeric is called *C. aromatica* and the domestic species is called *C. longa*.

Curcumin is extracted from the dried root of the rhizome *Curcuma Longa*. The process of extraction requires the raw material to be ground into powder, and washed with a suitable solvent that selectively extracts colouring matter. This process after distillation of the solvent yields an oleoresin with colouring matter content in the region of 25-35 percent along with volatile oils and other resinous extractives. The oleoresin so obtained is subjected to further washes using selective solvents that can extract the curcumin pigment from the oleoresin. This process yields a powdered, purified food colour, known as curcumin powder, with over 90 percent colouring matter content and very little volatile oil and other dry matter of natural origin. The selection of solvents is done with care to meet extractability and regulatory criteria. The following solvents are considered suitable:

- **Isopropanol**: In the curcumin manufacturing process isopropyl alcohol is used as a processing aid for purifying curcumin.

- **Ethyl acetate**: With a restriction placed on the use of chlorinated solvents, such as dichloroethane, it is found that ethyl acetate, owing to its polarity, is a reasonable replacement providing acceptable quality of product and commercially viable yields.

- **Acetone**: This is used as a solvent in the curcumin manufacturing process.
Carbon dioxide: This is not currently used in commercial production. However, it is listed in EC Directive 95/45/EC and has potential as a substitute for chlorinated solvents.

Methanol: This solvent is used occasionally as a processing aid for purification.

Ethanol: This solvent is used sparingly because curcumin is completely soluble in ethanol.

Chemical characterization

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has α-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%) (Kapoor, 1990). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%)(Ruby et al., 1995). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated (Vopel et al., 1990) (Figure 1). Curcumin was first isolated in 1815 and its chemical structure was determined by Roughley and Whiting in 1973. It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform.

The three principal colouring components of curcumin that are present in various proportions are all dicinnamoylmethane derivatives:

1) 1,7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione
   = diferuloylmethane
   (Chemical formula: C21H20O6; C.A.S. number: 458-37-7, Formula weight: 368)

2) 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione
   = p-hydroxycinnamoylferuloylmethane
   (Chemical formula: C20H18O5; C.A.S. number: 33171-16-3, Formula weight: 338)

3) 1,7-Bis-(4-hydroxyphenyl)-hepta-1,6-diene-3,5-dione
   = p, p-dihydroxydicinnamoylmethane
   (Chemical formula: C19H16O4; C.A.S. number: 33171-05-0, Formula weight: 308)
Physico-chemical properties

Curcumin is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, and soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (e.g. sodium dodecyl sulfate, cetylpyridinium bromide, gelatine, polysaccharides, polyethyleneglycol and cyclodextrins) have been reported (Tonnesen, 2002). In solutions the principal colouring components of curcumin exhibit keto-enol tautomerism and, depending on the solvent, up to 95 percent are in the enol form.

The kinetics of hydrolytic degradative reactions of compound 1 (diferuloylmethane) over the pH range 1-11 was studied using HPLC technique (Han et al., 2008). At pH <1, aqueous solutions of diferuloylmethane have a red colour which indicates the protonated form (H4A+). In the pH range 1-7, the majority of diferuloylmethane species are in the neutral form (H3A). Water solubility is very low in this pH range and solutions are yellow. At pH >7.5, the colour changes to red. The pKa values for the dissociation of the three acidic protons in compound 1 (forms H2A-, HA2- and A3-) have been determined to be 7.8, 8.5 and 9.0, respectively.

Curcumin biological property

Curcumin is stable in dry food. It is relatively stable to heat so it can be used in thermally treated foods. Reported reactions of curcumin with food constituents are bleaching of colour by sulfur dioxide at levels in excess of 100 ppm and formation of complexes with some salts (citrate, phthalate). The principal colouring
components of curcumin are apparently inert to chlorides, phosphates and bicarbonates (Tonnesen and Karlsen, 1985).

The principal colouring components of curcumin are effective antioxidants in food. The literature on the site of activity and the reaction mechanism(s) responsible for antioxidant effects are somewhat controversial, with most authors claiming that the antioxidant activity is due to the hydroxyl moiety (Sun et al., 2002; Barclay et al., 2000; Venkatesan and Rao, 2000), while others invoke involvement of double bonds and carbonyl groups, separately or together, with parahydroxy groups (Tonnesen and Greenhill, 1992; Sugiyama et al., 1996; Sreejayan and Rao, 1997). Studies showed that curcumin has a very powerful antioxidant effect (Sreejayan and Rao, 1994; Osawa et al., 1995).

Curcumin proved significantly more effective than other spices in its ability to prevent lipid peroxidation. Its antioxidant effect was eight times more powerful than vitamin E (Reddy and Lokesh, 1992) and it was significantly more effective in preventing lipid peroxide formation than the synthetic antioxidant BHT (Majeed et al., 2000). The antioxidant property of curcumin can prevent rancidity of foods and provide foodstuffs containing less oxidized fat or free radicals. The powerful antioxidation property of curcumin has an important role in keeping curry for a long time without it turning rancid.

The principal colouring components of curcumin scavenge free radicals at the cost of becoming weak free radicals themselves. According to one research report (Majeed et al., 2000) these "second hand" free radicals are unreactive and short-lived products (unlike those of synthetic phenolics, e.g., BHT or BHA) and do not pose a health hazard.

It is reported that in organic solvents and in some micellar solubilized systems the principal colouring components of curcumin act as photo-sensitizers of singlet oxygen, superoxide and free radicals (Chignell et al., 1994). This ability can have a destabilizing effect on curcumin-containing products. On the other hand, light-induced oxidation can be applied in systems with biological destructive behaviour; e.g. in the killing of bacteria (Liang et al., 2008).
Turmeric powder, curcumin and its derivatives and many other extracts from the rhizomes were found to be bioactive (Table 1). The structures of some of these compounds (Araujo and Leon, 2001) are presented in Figure 1. Turmeric powder has healing effect on both aseptic and septic wounds in rats and rabbits (Panchatcharam et al., 2006). It also shows adjuvant chemoprotection in experimental forestomach and oral cancer models of Swiss mice and Syrian golden hamsters (Azuine and Bhide, 1994). Curcumin also increases mucin secretion in rabbits (Lee et al., 2003). Curcumin, the ethanol extract of the rhizomes, sodium curcuminate, [feruloyl-(4-hydroxycinnamoyl)-methane] (FHM) and [bis-(4-hydroxycinnamoyl) - methane] (BHM) and their derivatives, have high antiinflammatory activity against carrageenin-induced rat paw oedema (Ammon et al., 1993). It elevates the activity of pancreatic lipase, amylase, trypsin and chymotrypsin (Platel and Srinivasan, 2000) Curcumin protects isoproterenol-induced myocardial infarction in rats (Nirmala and Puvanakrishnan, 1996). Curcumin, FHM and BHM also have anticoagulant activity (Krishnaswamy, 2008). Curcumin and an etherextract of C. longa have hypolipemic action in rats and lower cholesterol, fatty acids and triglycerides in alcohol induced toxicity (Rukkumani et al., 2003). Curcumin is also reported to have antibacterial (Kumar et al., 2001), antiamoebic and antiHIV activities. Curcumin also shows antioxidant activity (Pulla, 1994; Unnikrishnan and Rao, 1995; Song et al., 2001; Phan et al., 2001). It also shows antitumour (Huang et al., 1988; Bhaumik, 1993; Surh et al., 2001) and anticarcinogenic (Kuo et al., 1996; Goel et al., 2001; Shao et al., 2002; Choudhuri et al., 2002) activities. The volatile oil of C. longa shows anti-inflammatory (Chandra and Gupta, 1972), antibacterial (Lutomski, 1974) and antifungal (Banerjee and Nigam, 1978) activities. The petroleum ether extract longa is reported to have antiinflammatory activity (Arora et al., 1971). Petroleum ether and aqueous extracts have 100% antifertility effects in rats (Garg, 1974). Fifty per cent ethanolic extract of C. longa shows hypolipemic action (Dixit et al., 1988) in rats. Ethanolic extract also possesses antitumour activity (Kuttan et al., 1985). Alcoholic extract and sodium curcuminate can also offer antibacterial activity (Bhavani Shankar and Sreenivasa, 1979). The crude ether and chloroform extracts of C. longa stem are also reported to have antifungal effects (Misra et al., 1977). A C. longa fraction containing ar-turmerone has potent antivenom activity (Ferreira et al., 1992).
Pharmacological action of curcumin

Effect on gastrointestinal system Stomach: Turmeric powder has beneficial effect on the stomach. It increases mucin secretion in rabbits and may thus act as gastroprotectant against irritants (Lee et al., 2003). However, controversy exists regarding antiulcer activity of curcumin. Both antiulcer and ulcerogenic (Mahattanadul et al., 2009) effects of curcumin have been reported but detailed studies are still lacking. Curcumin has been shown to protect the stomach from ulcerogenic effects of phenylbutazone in guinea pigs at 50 mg/kg dose (Sinha et al., 1974). It also protects from 5-hydroxytryptamine- induced ulceration at 20 mg/kg dose. However, when 0.5% curcumin was used, it failed to protect against histamine-induced ulcers (Ammon and Wahl, 1991). In fact, at higher doses of 50 mg/kg and 100 mg/kg, it produces ulcers in rats (Gupta et al., 1980). Though the mechanism is not yet clear, an increase in the gastric acid and/or pepsin secretion and reduction in mucin content have been implicated in the induction of gastric ulcer (Cream et al., 1974). Recent studies in our laboratory indicate that curcumin can block indomethacin, ethanol and stress-induced gastric ulcer and can also prevent pylorus-ligation-induced acid secretion in rats. The antiulcer effect is mediated by scavenging of reactive oxygen species by curcumin.

Intestine: Curcumin has some good effects on the intestine also. Antispasmodic activity of sodium curcuminate was observed in isolated guinea pig ileum (Srihari et al., 1982). Antiflatulent activity was also observed in both in vivo and in vitro experiments in rats. Curcumin also enhances intestinal lipase, sucrase and maltase activity (Platel and Srinivasan, 1996).

Liver: Curcumin and its analogues have protective activity in cultured rat hepatocytes against carbon tetrachloride, D-galactosamine, peroxide and ionophore-induced toxicity (Song et al., 2001; Kang et al., 2002). Curcumin also protects against diethylnitrosamine and 2-acetylaminofluorine-induced altered hepatic foci development (Shukla and Arora, 2003a). Increased bile production was reported in dogs by both curcumin and essential oil of C. longa (Deters et al., 2000).

Pancreas: 1-phenyl-1-hydroxy-n-pentane, a synthetic derivative of p-tolylmethylcarbinol (an ingredient of C. longa) increases plasma secretion and bicarbonate levels (Chey et al., 1983). Curcumin also increases the activity of pancreatic lipase, amylase, trypsin and chymotrypsin (Platel and Srinivasan, 2000).
Effect on cardiovascular system: Curcumin decreases the severity of pathological changes and thus protects from damage caused by myocardial infarction (Nirmala and Puvanakrishnan, 1996). Curcumin improves Ca\(^{2+}\) transport and its slippage from the cardiac muscle sarcoplasmic reticulum, thereby raising the possibility of pharmacological interventions to correct the defective Ca\(^{2+}\) homeostasis in the cardiac muscle (Sumbilla et al., 2002). Curcumin has significant hypocholesteremic effect in hypercholesteremic rats (Patil et al., 1971).

Effect on nervous system: Curcumin and manganese complex of curcumin offer protective action against vascular dementia by exerting antioxidant activity (Vajragupta et al., 2003; Thiyagarajan and Sharma, 2004).

Effect on lipid metabolism: Curcumin reduces low density lipoprotein and very low density lipoprotein significantly in plasma and total cholesterol level in liver along with an increase of \(\alpha\)-tocopherol level in rat plasma, suggesting \textit{in vivo} interaction between curcumin and \(\alpha\)-tocopherol that may increase the bioavailability of vitamin E and decrease cholesterol levels (Kamal-Eldin et al., 2000). Curcumin binds with egg and soy-phosphatidylcholine, which in turn binds divalent metal ions to offer antioxidant activity (Began et al., 1999). The increase in fatty acid content after ethanol-induced liver damage is significantly decreased by curcumin treatment and arachidonic acid level is increased (Akrishnan and Menon, 2001).

Anti-inflammatory activity: Curcumin is effective against carrageenin-induced oedema in rats and mice (Brouet and Ohshima, 1995). The natural analogues of curcumin, viz. FHM and BHM, are also potent anti-inflammatory agents (Gautam and Jachak, 2009). The volatile oil and also the petroleum ether, alcohol and water extracts of \textit{C. longa} show anti-inflammatory effects has also been recently reported (Itokawa et al., 2008). The antirheumatic activity of curcumin has also been established in patients who showed significant improvement of symptoms after administration of curcumin (Deodhar et al., 1980). That curcumin stimulates stress-induced expression of stress proteins and may act in a way similar to indomethacin and salicylate, has recently been reported (Kato et al., 1998). Curcumin offers antiinflammatory effect through inhibition of NFkB activation (Singh and Aggarwal, 1995). Curcumin has also been shown to reduce the TNF-\(\alpha\)-induced expression of the tissue factor gene in bovine aortic-endothelial cells by repressing
activation of both AP-1 and NFκB (Bierhous et al., 1997). The anti-inflammatory role of curcumin is also mediated through downregulation of cyclooxygenase-2 and inducible nitric oxide synthetase through suppression of NFκB activation (Surh et al., 2001). Curcumin also enhances wound-healing in diabetic rats and mice (Sidhu et al., 1999), and in H2O2-induced damage in human keratinocytes and fibroblasts (Phan et al., 2001).

**Antioxidant effect:** The antioxidant activity of curcumin was reported (Sharma, 1976) as early as 1975. It acts as a scavenger of oxygen free radicals, (Subramanian et al., 1994). It can protect haemoglobin from oxidation (Unnikrishnan and Rao, 1995). *In vitro*, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H2O2 and nitrite radical generation by activated macrophages, which play an important role in inflammation (Joe and Lokesh, 1994). Curcumin also lowers the production of ROS *in vivo* (Joe and Lokesh, 1994). Its derivatives, demethoxycurcumin and bis-demethoxycurcumin also have antioxidant effect (Unnikrishnan and Rao, 1995). Curcumin exerts powerful inhibitory effect against H2O2 induced damage in human keratinocytes and fibroblasts and in NG 108-15 cells (Mahakunakorn et al., 2003). Curcumin reduces oxidized proteins in amyloid pathology in Alzheimer transgenic mice (Lim et al., 2001a). It also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Pulla Reddy and Lokesh, 1994). This is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (Pulla Reddy and Lokesh, 1992).

Recently, it has been observed that curcumin prevents oxidative damage during indomethacin-induced gastric lesion not only by blocking inactivation of gastric peroxidase, but also by direct scavenging of H2O2 and ·OH. Since ROS have been implicated in the development of various pathological conditions (Bandyopadhyay et al., 1999; Halliwell, 1998; Halliwell and Gutteridge, 1990), curcumin has the potential to control these diseases through its potent antioxidant activity. Contradictory to the above-mentioned antioxidant effect, curcumin has pro-oxidant activity. Kelly et al. (2001) reported that curcumin not only failed to prevent single-strand DNA breaks by H2O2, but also caused DNA damage. As this damage was prevented by antioxidant α-tocopherol, the pro-oxidant role of
Curcumin has been proved. Curcumin also causes oxidative damage of rat hepatocytes by oxidizing glutathione and of human erythrocyte by oxidizing oxyhaemoglobin, thereby causing haemolysis (Galati et al., 2002). The prooxidant activity appears to be mediated through generation of phenoxyl radical of curcumin by peroxidase–H2O2 system, which cooxidizes cellular glutathione or NADH, accompanied by O2· uptake to form ROS (Galati et al., 2002).

The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of \( b \)-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant (Figure 1) (Sreejayan, 1994; Masuda et al., 2001). Generally, the nonenzymatic antioxidant process of the phenolic material is thought to be mediated through the following two stages:

\[
S - \text{OO}^\circ + \text{AH} \rightarrow \text{SOOH} + \text{A}^\circ, \\
\text{A}^\circ + \text{X}^\circ \rightarrow \text{Nonradical materials,}
\]

where \( S \) is the substance oxidized, \( \text{AH} \) is the phenolic antioxidant, \( \text{A}^\circ \) is the antioxidant radical and \( \text{X}^\circ \) is another radical species or the same species as \( \text{A}^\circ \). \( \text{A}^\circ \) and \( \text{X}^\circ \) dimerize to form the non-radical product. Masuda et al. (2001) further studied the antioxidant mechanism of curcumin using linoleate as an oxidizable polyunsaturated lipid and proposed that the mechanism involves oxidative coupling reaction at the 3\( \gamma \)position of the curcumin with the lipid and a subsequent intramolecular Diels–Alder reaction.

**Antigenotoxic effect and Anticarcinogenic effect**

Curcumin acts as a potent antigenotoxic compound. Among various mechanisms, induction of apoptosis plays an important role in its anticarcinogenic effect. It induces apoptosis and inhibits cell-cycle progression, both of which are instrumental in preventing cancerous cell growth in rat aortic smooth muscle cells (Chen and Huang, 1998b). The antiproliferative effect is mediated partly through inhibition of protein tyrosine kinase and c-myc mRNA expression and the apoptotic effect may partly be mediated through inhibition of protein tyrosine kinase, protein kinase C, c-myc mRNA expression and bcl-2mRNA expression (Chen and Huang, 1998b). Curcumin induces apoptotic cell death by DNA-damage in human cancer cell lines, TK-10, MCF-7 and UACC-62 by acting as topoisomerase II poison (Martin-Cordero et al., 2003).
Recently, curcumin has been shown to cause apoptosis in mouse neuro cells by impairing the ubiquitin–proteasome system through the mitochondrial pathway (Jana et al., 2004). Curcumin causes rapid decrease in mitochondrial membrane potential and release of cytochrome c to activate caspase 9 and caspase 3 for apoptotic cell death (Jana et al., 2004). Recently, an interesting observation was made regarding curcumin-induced apoptosis in human colon cancer cell and role of heat shock proteins (hsp) thereon (Rashmi et al., 2004). In this study, SW480 cells were transfected with hsp 70 cDNA in either the sense or antisense orientation and stable clones were selected and tested for their sensitivity to curcumin. Curcumin was found to be ineffective to cause apoptosis in cells having hsp 70, while cells harbouring antisense hsp 70 were highly sensitive to apoptosis by curcumin as measured by nuclear condensation, mitochondrial transmembrane potential, release of cytochrome c, activation of caspase 3 and caspase 9 and other parameters for apoptosis (Rashmi et al., 2004). Expression of glutathione S-transferase P1-1 (GSTP1-1) is correlated to carcinogenesis and curcumin has been shown to induce apoptosis in K562 leukaemia cells by inhibiting the expression of GSTP1-1 at transcription level (Duvoix et al., 2003). The mechanism of curcumin-induced apoptosis has also been studied in Caki cells, where curcumin causes apoptosis through downregulation of Bcl-XL and IAP, release of cytochrome c and inhibition of Akt, which are markedly blocked by N-acetylcysteine, indicating a role of ROS in curcumin induced cell death (Woo et al., 2003). In LNCaP prostate cancer cells, curcumin induces apoptosis by enhancing tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) 97. The combined treatment of the cell with curcumin and TRAIL induces DNA fragmentation, cleavage of procaspase 3, 8 and 9, truncation of Bid and release of cytochrome c from mitochondria, indicating involvement of both external receptor-mediated and internal chemical-induced apoptosis in these cells (Deeb et al., 2003). In colorectal carcinoma cell line, curcumin delays apoptosis along with the arrest of cell cycle at G1 phase (Chen et al., 1996). Curcumin also reduces P53 gene expression, which is accompanied with the induction of HSP-70 gene through initial depletion (Chen et al., 1996) of intracellular Ca2+. Curcumin also produces nonselective inhibition of proliferation in several leukaemia, nontransformed haematopoietic progenitor cells and fibroblast cell lines (Gautam et al., 1998). That curcumin induces apoptosis and large-scale DNA fragmentation has also been observed in Vγ9Vδ2 T cells through inhibition.
of isopentenyl pyrophosphate-induced NFκB activation, proliferation and chemokine production (Cipriani et al., 2001). Curcumin induces apoptosis in human leukaemia HL-60 cells, which is blocked by some antioxidants (Kuo et al., 1996). Colon carcinoma is also prevented by curcumin through arrest of cell-cycle progression independent of inhibition of prostaglandin synthesis (Hanif et al., 1997). Curcumin suppresses human breast carcinoma through multiple pathways. Its antiproliferative effect is estrogen-dependent in ER (estrogen receptor)-positive MCF-7 cells and estrogen-independent in ER-negative MDA-MB-231 cells (Shao et al., 2002). Curcumin also downregulates matrix metalloproteinase (MMP)-2 and upregulates tissue inhibitor of metalloproteinase (TIMP)-1, two common effector molecules involved in cell invasion (Shao et al., 2002). It also induces apoptosis through P53-dependent Bax induction in human breast cancer cells (Choudhuri et al., 2002). However, curcumin affects different cell lines differently. Whereas leukaemia, breast, colon, hepatocellular and ovarian carcinoma cells undergo apoptosis in the presence of curcumin, lung, prostate, kidney, cervix and CNS malignancies and melanoma cells show resistance to cytotoxic effect of curcumin (Khar et al., 2001).

**Pro/antimutagenic activity**

Curcumin exerts both pro- and antimutagenic effects. At 100 and 200 mg/kg body wt doses, curcumin has been shown to reduce the number of aberrant cells in cyclophosphamid induced chromosomal aberration in Wistar rats. Turmeric also prevents mutation in urethane (a powerful mutagen) models (Hamss et al., 1999). Contradictory reports also exist. Curcumin and turmeric enhance γ-radiation-induced chromosome aberration in Chinese hamster ovary (Araujo et al., 1999). Curcumin has also been shown to be non-protective against hexavalent chromium-induced DNA strand break. In fact, the total effect of chromium and curcumin is additive in causing DNA breaks in human lymphocytes and gastric mucosal cells (Blasiak et al., 1999).
Andrographis paniculata

TAXONOMICAL CLASSIFICATION

Kingdom : Plantae, Plants
Division : Angiosperma
Class : Dicotyledonae
Sub class : Gamopetalae
Order : Personales
Family : Acanthaceae
Genus : Andrographis
Species : paniculata

*Andrographis paniculata* (Burm.f.) Wall ex. Nees belongs to the family *Acanthaceae*. It is an erect branched annual herb, 0.3-0.9m in height with quadrangular branches. Leaves are simple, lanceolate, acute at both ends, glabrous, with 4-6 pairs of main nerves. Flowers are small, pale but blotched and spotted with brown and purple distant in lax spreading axillary and terminal racemes or panicles. Calyx-lobes are glandular pubescent with anthers bearded at the base. Fruits are linear capsules and acute at both ends. Seeds are numerous, yellowish brown and sub-quadrate (Warrier et al., 1993). Another species of Andrographis is *A. echioides* (Linn.) Nees. It is found in the warmer parts of India. The plant is a febrifuge and diuretic. It contains flavone-echioidinin and its glucoside-echioidin (Husain et al., 1992).

**Description**

A herbaceous annual, erect, up to 1m high; stem acutely quadrangular, much branched. Leaves simple, opposite, lanceolate, glabrous, 2-12cm long, 1-3cm wide; apex acute; margin entire, slightly undulate, upper leaves often bractiform; petiole short. Inflorescence patent, terminal and axillary in panicle, 10-30mm long; bract small; pedicel short. Calyx 5-particle, small, linear. Corolla tube narrow, about 6 mm long; limb longer than the tube, bilabiate; upper lip oblong, white with a yellowish top; lower lip broadly cuneate, 3-lobed, white with violet markings. Stamens 2, inserted in the throat and far exserted; anther basally bearded. Superior ovary, 2-celled; style far exserted. Capsule erect, linear-oblong, 1-2 cm long and 2-
5 mm wide, compressed, longitudinally furrowed on broad faces, acute at both ends, thinly glandular-hairy. Seeds small, subquadrate (ASEAN, 1993).

**Major chemical constituents**

The major constituents are diterpene lactones (free and in glycosidic forms) including andrographolide, deoxyandrographolide, 11, 12-didehydro-14-deoxyandrographolide, neoandrographolide, andrographiside, deoxyandrographiside and andropanoside (ASEAN, 1993; Blaschek, 1998). The structures of andrographolide and related diterpene lactones are presented below.

![Structures of diterpene lactones](image)

**Properties and Activity**

Leaves contain two bitter substances lactone “andrographolid” and “kalmeghin”. The ash contains sodium chloride and potassium salts. Plant is very rich in chlorophyte. Kalmeghin is the active principle that contains 0.6% alkaloid of the crude plant. The plant contains diterpenoids, andrographolide, 14-deoxy-11-oxo-andrographolide, 14-deoxy-11, 12-dihydroandrographolide, 14-deoxy andrographolide and neoandrographolide (Chen et al., 2006). The roots give flavones-apigenin-7, 4-dio-O-methyl ether, 5-hydroxy-7, 8, 2', 3'-tetramethoxyflavone, andrographin and panicolin and α-sitosterol (Koteswara et al., 2004). Leaves contain homoandrographolid, rographosterol and andrographone.
The plant is vulnerary, antipyretic, antiperiodic, antiinflammatory, expectorant, depurative, sudorific, anthelmintic, digestive, stomachic, tonic, febrifuge and chologogue. The plant is antifungal, antityphoid, hepatoprotective, antidiabetic and cholinergic. Shoot is antibacterial and leaf is hypotensive (Yoopan et al., 2007). This is used for the inflammation of the respiratory tract. In China, researchers have isolated the andrographolide from which soluble derivative such as 14-deoxy-11, 12-dehydro-andrographolide which forms the subject of current pharmacological and clinical studies. Apigenin 7,4'-O-dimethyl ether isolated from A. paniculata exhibits dose dependent, antiulcer activity in shay rat, histamine induced ulcer in guinea pigs and aspirin induced ulcers in rats. A crude substance isolated from methanolic extract of leaves has shown hypotensive activity. Pretreatment of rats with leaf (500mg/kg) or andrographolide (5mg/kg) orally prevented the carbon tetrachloride induced increase of blood serum levels of glutamate-oxaloacetate transaminase in liver and prevented hepatocellular membrane.

Antibacterial activity

An ethanol extract of the leaves inhibited the growth in vitro of Escherichia coli and Staphylococcus aureus (Jiang et al., 2009). However, no in vitro antibacterial activity was observed when dried powder from the aerial parts was tested against E. coli, Staphylococcus aureus, Salmonella typhi or Shigella species (Leelarasamee et al., 1990).

Anti-human immunodeficiency virus (HIV) activity

Aqueous extracts of the leaves inhibited HIV-1 infection and replication in the lymphoid cell line MOLT-4 (Yao et al. 1992). An ether extract of the aerial parts reduced the percentage of HIV antigen-positive H9 cells (Reddy et al., 2005). Dehydroandrographolide inhibited HIV-1 and HIV-1 (UCD123) infection of H9 cells at 1.6mg/ml and 50mg/ml, respectively, and also inhibited HIV-1 infection of human lymphocytes at 50mg/ml (Chang et al., 1991). A methanol extract of the leaves suppressed syncytia formation in co-cultures of uninfected and HIV-1-infected MOLT cells (median effective dose [ED50] 70mg/ml) (Otake et al., 1995).
**Immunostimulatory activity**

Intragastric administration of an ethanol extract of the aerial parts (25mg/kg body weight) or purified andrographolides (1 mg/kg body weight) to mice stimulated antibody production and the delayed-type hypersensitivity response to sheep red blood cells (Puri et al., 1993). The extract also stimulated a non-specific immune response in mice, measured by macrophage migration index, phagocytosis of [14C] leucine-labelled *E. coli*, and proliferation of splenic lymphocytes (Puri et al., 1993). The extract was more effective than either andrographolide or neoandrographolide alone, suggesting that other constituents may be involved in the immunostimulant response (Puri et al., 1993).

**Antipyretic activity**

Intragastric administration of an ethanol extract of the aerial parts (500mg/kg body weight) to rats decreased yeast-induced pyrexia (Vedavathy and Rao, 1991). The extract was reported to be as effective as 200 mg/kg body weight of aspirin, and no toxicity was observed at doses up to 600mg/kg body weight (Vedavathy and Rao, 1991). Intragastric administration of andrographolide (100mg/kg body weight) to mice decreased brewer’s yeast-induced pyrexia (Madav et al., 1995). Intragastric administration of deoxyandrographolide, andrographolide, neoandrographolide or 11, 12-didehydro-14-deoxyandrographolide (100mg/kg body weight) to mice, rats or rabbits reduced pyrexia induced by 2,4-dinitrophenol or endotoxins (Deng et al., 1982).

**Antidiarrhoeal activity**

Herba Andrographidis has antidiarrhoeal activity in situ (Gupta et al., 1993; Gupta et al., 1990). An ethanol, chloroform or 1-butanol extract of the aerial parts (300mg/ml) inhibited the *E. coli* enterotoxin-induced secretory response which causes a diarrhoeal syndrome in the rabbit and guinea-pig ileal loop assay (Gupta et al., 1993; Gupta et al., 1990). However, an aqueous extract of the aerial parts was not active (Gupta et al., 1990). The constituent diterpene lactones, andrographolide and neoandrographolide, exhibited potent antisecretory activity in vivo against *E. coli* enterotoxin-induced diarrhoea (Gupta et al., 1990). Andrographolide (1 mg per loop) was as active as loperamide when tested against heat-labile *E. coli* enterotoxin-induced diarrhoea and more effective than
loperamide when tested against heat-stable *E. coli* enterotoxin-induced diarrhea (Gupta et al., 1990). Neoandrographolide (1 mg per loop) was as effective as loperamide when tested against heat-labile *E. coli* enterotoxin-induced diarrhoea and slightly less active than loperamide when tested against heat-stable *E. coli* enterotoxin-induced diarrhoea (Gupta et al., 1990). The mechanism of action involves inhibition of the intestinal secretory response induced by heat-labile *E. coli* enterotoxins, which are known to act through the stimulation of adenylate cyclase, and by inhibition of the secretion induced by heat-stable *E. coli* enterotoxins, which act through the activation of guanylate cyclase (Gupta et al., 1993). Incubation of murine macrophages with andrographolide (1–50 mmol/l) inhibited bacterial endotoxin-induced nitrite accumulation in a concentration- and time dependent manner. Western blot analysis demonstrated that andrographolide inhibited the expression of an inducible isoform of nitric oxide synthase linked to endotoxin-induced circulatory shock (Chiou et al., 1998).

**Anti-inflammatory activity**

Intragastric administration of deoxyandrographolide, andrographolide, neoandrographolide or 11,12-didehydrodeoxyandrographolide to mice inhibited the increase in cutaneous or peritoneal capillary permeability induced by xylene or acetic acid, and reduced acute exudation in Selye granulocysts treated with croton oil. 11, 12-Didehydrodeoxyandrographolide had the most potent anti-inflammatory activity in vivo.

**Antimalarial activity**

A 50% ethanol extract of the aerial parts inhibited the growth of *Plasmodium berghei* both *in vitro* (100 mg/ml) and in mice after intragastric administration (1 g/kg body weight) (Misra et al., 1991b). Intragastric administration of a 1-butanol, chloroform or ethanol–water extract of the aerial parts to *Mastomys natalensis* inhibited the growth of *P. berghei* at doses of 1–2 g/kg body weight (Misra et al., 1992). Andrographolide (5 mg/kg body weight) and neoandrographolide (2.5mg/kg body weight) were also effective when administered by gastric lavage (Misra et al., 1992).
**Antivenom activity**

Intraperitoneal injection of an ethanol extract of the aerial parts (25 g/kg body weight) to mice poisoned with cobra venom markedly delayed the occurrence of respiratory failure and death (Nazimudeen, 1978). The same extract induced contractions in guinea-pig ileum at concentrations of 2 mg/ml. The contractions were enhanced by physostigmine and blocked by atropine, but were unchanged by antihistamines (Nazimudeen et al., 1978). These data suggest that extracts of the aerial parts do not modify the activity of the nicotinic receptors but produce significant muscarinic activity, which accounts for its antivenom effects (Nazimudeen et al., 1978).

**Antihepatotoxic activity**

The aerial parts and their constituent andrographolide have antihepatotoxic activity *in vitro* and *in vivo* (Chander et al., 1995; Bhaumik and Sharma, 1993; Kapil, 1993; Visen et al., 1993; Saraswat et al., 1995). Intraperitoneal administration of a methanol extract of the aerial parts (861.3 mg/kg body weight) to mice reduced hepatotoxicity induced by carbon tetrachloride (CCl₄), and reversed CCl₄-induced histopathological changes in the liver (Handa and Sharma, 1990). Intraperitoneal administration of andrographolide (100 mg/kg body weight) to mice inhibited the CCl₄-induced increase in the activity of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, bilirubin and hepatic triglycerides (Handa and Sharma, 1990). Intraperitoneal administration of a methanol extract of the aerial parts (500 mg/kg body weight) to rats also suppressed the CCl₄-induced increase in the activity of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase and bilirubin (Sharma et al., 1991). Intragastric administration of an aqueous extract of the aerial parts (500mg/kg body weight) to ethanol-treated rats decreased the activity of serum transaminases and suppressed histopathological changes in the liver (Pramyothin, 1993). Andrographolide, the major antihepatotoxic component of the plant, exerted a pronounced protective effect in rats against hepatotoxicity induced by CCl₄ (Kapil, 1993), Dgalactosamine (Saraswat et al., 1995), paracetamol (Visen et al., 1993) and ethanol (Pramyothin, 1993). Andrographolide was more effective than silymarin, the standard hepatoprotective agent (Kapil, 1993; Visen et al., 1993).
Antioxidant effects

Methanolic extract of Andrographis paniculata was found to inhibit formation of oxygen derived free radicals such as superoxide, hydroxyl radicals, lipid peroxidation and nitric oxide in \textit{in vitro} system. \textit{In vivo} studies of BALB/c mice, administration of Andrographis paniculata extract produced complete inhibition of carageenan induced inflammation compared with controls. Researchers are hoping these activities lead to multiple benefits of Andrographis in health (Sheeja et al., 2006). Another study of andrographolide showed that it suppressed nitric oxide (NO) production in a concentration-dependent manner in the concentration range from 0.1 to 100 \( \mu \text{M} \) and their IC\textsubscript{50} values were 7.9 and 35.5 \( \mu \text{M} \). Neoandrographolide also suppressed NO production by 35 and 40\% when the macrophages were collected after oral administration of neoandrographolide at doses of 5 and 25 mg/kg/d. However, andrographolide did not reduce NO production on oral administration at the same doses (Batkhuu et al., 2002).

In the light of above information, these plant products are rich in compounds having antioxidant, antimutagenic and other properties. Hence, curcumin and andrographolides of herbal origin have been chosen to investigate amelioration on \textit{in vivo} and \textit{in vitro} effects induced by nickel (II) ions.