Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Nickel

(Request N° EFSA-Q-2003-018)

(adopted on 25 January 2005 by written procedure)

SUMMARY

The European Food Safety Authority is asked to derive an upper level for the intake of nickel from food that is unlikely to pose a risk of adverse health effects.

Nickel has not been shown to be essential for humans. Orally ingested nickel salts can cause adverse effects on kidneys, spleen, lungs and the myeloid system in experimental animals. Furthermore, perinatal mortality was reported to be increased in the offspring of female rats ingesting nickel salts, even at the lowest administered dose (1.3 mg nickel/kg body weight/day). While there is evidence that inhaled nickel salts are carcinogenic in rodents and humans, orally ingested nickel salts have not been shown to be carcinogenic; however the data presently available are very limited.

Individuals sensitised to nickel through dermal contact and who have allergic contact dermatitis (estimated to be up to 15% of women but frequently undiagnosed) develop hand eczema from oral, as well as dermal, exposure to nickel salts. Oral intakes of nickel as low as about 500 μg/day (about 8 μg/kg body weight/day) have been reported to aggravate hand eczema in nickel sensitised subjects.

In the absence of adequate dose-response data for these effects, it is not possible to establish a tolerable upper intake level.

The intake of nickel from the average diet is estimated to be about 150 μg/day (about 2.5 μg/kg body weight/day), but may reach 900 μg/day (about 15 μg/kg body weight/day) or more, when large amounts of food items with high nickel contents are consumed. In addition, first-run drinking water, which may contain up to 1000 μg/L, and leaching from kitchen utensils into food may also contribute to nickel intake. Intakes of 150 and 900 μg/day are about 500 and 90-fold lower, respectively, than the lowest dose reported to cause adverse effects in rats. Average intakes from food are about one third of the lowest intake reported to aggravate hand eczema in nickel sensitised subjects.

KEY WORDS

Nickel, tolerable upper intake level, food safety.

BACKGROUND

In 2002, the European Parliament and the Council adopted Directive 2002/46/EC[1] related to food supplements containing vitamins and minerals.

In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission's legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. The SCF did not have sufficient time to adopt opinions for the following vitamins and minerals: vitamin C, chloride, fluoride, iron, phosphorus, potassium and sodium. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested that boron, nickel, silicon, vanadium and tin should be allowed to be used in food supplements. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

TERMS OF REFERENCE

With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Nickel occurs naturally in soil, water, plants and animals. In its compounds, it has normally the valency state of +2, but valency states of 0, +1, +3, and +4 also exist.

[1] Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements. OJ L 183. 12.7.2002, p. 51.

2. NUTRITIONAL BACKGROUND

2.1 Food levels and dietary intake

The nickel concentration is highest in cocoa (8.2-12 mg/kg), soya beans (4.7-5.9 mg/kg), oatmeal (0.33-4.8 mg/kg), hazelnuts (0.66-2.3 mg/kg), almonds (1.2-1.3 mg/kg) and legumes. The intake from a Danish average diet was estimated to be 150 μg/person/day, but may reach 900 μg/person/day or more, when large amounts of food items with high nickel contents not included in the Danish average diet are consumed (Flyvholm *et al.*, 1984). The nickel content of a typical Swedish diet assessed by analysis of market baskets was on average 82 μg/day (Becker and Kumpulainen, 1991), while it was 115 μg/day (range 70-170 μg/day) in duplicate 7-day diets collected from 15 women in the Stockholm area (Jorhem *et al*., 1998). Other estimates of 100-146 μg/day (Pennington and Jones, 1987) and 20-406 μg/day (Dabeka and McKenzie, 1995) for the average nickel intake by adults are in the same range.

The maximum nickel content in running drinking water from different water works in the greater Copenhagen area was 35 μg/L (Andersen *et al.*, 1983). Similarly, maximum concentrations in drinking and mineral water found in Germany were 34 μg/L and 31 μg/L, respectively (Scheller *et al.*, 1988). After remaining in the tap for 8 hours or overnight, however, levels of 490 μg/L (Andersen *et al.*, 1983) and 1000 μg/L (WHO, 1996) have been reported, respectively.

Release from kitchen utensils can increase the nickel content of food. The average contribution of this source to the oral intake of nickel is unknown, but could augment dietary exposure by as much as 1 mg/day (Grandjean *et al.*, 1989, quoted by IARC, 1990).

2.2 Nutritional requirements and recommendations

Nutritional requirements or recommended dietary allowances for nickel have not been established. The SCF stated explicitly that the data were not sufficiently conclusive to justify setting any recommended intakes (SCF, 1993).

2.3 Deficiency

In some species, signs of deficiency have been observed. In rats, nickel deficiency was found to be associated with growth retardation, impaired reproduction function and lower haemoglobin levels. In humans, however, nickel deficiency has not been demonstrated.

3. BIOLOGICAL CONSIDERATIONS

3.1 Function

Nickel is essential for the catalytic activity of some plant and bacterial enzymes. It is said to influence iron absorption and metabolism and the haemopoietic process. However, biochemical functions of nickel have not been demonstrated in humans and higher animals.

3.2 Absorption, distribution, metabolism and excretion

The rate of absorption of nickel salts can be quite high in the fasting state, but is reduced significantly in the presence of food, such as milk, coffee, tea and orange juice (Solomons *et al.*, 1982). After an overnight fast, the absorbed nickel averaged 27±17% of a dose of nickel sulphate ingested in water versus 0.7±0.4% of the same dose ingested in food (Sunderman *et al.*, 1989). In a study with four healthy adults who were given after an overnight fast a dose of 10 μg/kg body weight of the stable isotope ^{62}Ni, and using ICP-MS, the absorbed amount was 29 to 40% (mean 33.4) of the dose. Plasma nickel levels rose rapidly within 1.5 to 2.5 hours to peak levels of 15 to 20 μg/L and declined by a factor of >10 during the next 3-4 days. Between 51 and 82% of the absorbed amount was excreted in urine over five days, but 34.8 ± 13.4% of the absorbed amount was retained after five days (Patriarca *et al.*, 1997). Nickel binds to albumin, histidine and α_2-macroglobulin and is widely distributed in the organism. Transplacental transfer has been demonstrated in rodents. Absorbed nickel is mainly excreted in the urine, but to a minor extent also in bile and sweat. It is secreted into human milk (Heseker, 2000).

3.3 Normal levels in human tissues and fluid

The total nickel content of the human body is estimated to be 0.5 mg (Heseker, 2000). Highest levels in human tissues occur in lungs, thyroid, adrenals, and kidneys with concentrations of 173, 141, 132 and 62 μg/kg dry weight, respectively (Rezuke *et al.*, 1987). In non-occupationally exposed men, the mean concentration of nickel in whole blood and serum is in the range of 1-5 μg/L and in urine less than 10 μg/L (ECETOC, 1989).

4. HAZARD IDENTIFICATION

This section focuses on the oral toxicity of nickel compounds and on studies particularly important for the risk assessment of nickel in food. It does not consider data for other routes with the exception of carcinogenicity. Data on hazards of inhalation and dermal exposure including aspects of occupational medicine have been reviewed elsewhere (Coogan *et al.*, 1989; ECETOC, 1989; IARC, 1990; WHO, 1991; WHO, 1996).

4.1 Acute toxicity

The acute oral toxicity of nickel compounds depends on their solubility. The soluble nickel chloride and nickel sulphate were found to have LD_{50} values in rats, equivalent to 42-129 mg nickel/kg body weight (ECETOC, 1989).

4.2 Subacute/subchronic toxicity

30 male rats were administered by gavage 25 mg nickel sulphate/kg body weight/day equivalent to 9.5 mg nickel/kg body weight/day over 120 days (10 males as controls). Testis, livers and kidneys were examined histologically and histochemically. In treated animals severe lesions in the germ cells particularly in spermiogenesis were observed. Changes in liver and kidneys were detected only rarely (Waltschewa *et al.*, 1972).

According to Fairhurst and Illing (1987) quoted and reviewed by ECETOC (1989), a number of oral subacute and subchronic studies have been performed with nickel carbonate, chloride,

and sulphate mainly in rats. In the most relevant of these studies the following effects were found in rats: (a) decreased body weight gain and slightly lowered haemoglobin levels (nickel chloride in the diet, equivalent to 0 and 20 mg nickel/kg diet, given for 42 days), (b) reduced body weight gain and elevated serum glucose at all dose levels (nickel chloride in drinking water, equivalent to 0, 2.5, 5 und 10 mg nickel/L, given for 28 days) and (c) lack of weight gain and extensive proliferation of lymphoid cells and histiocytes as well as micronecrosis in the intestine at the highest dose level (nickel sulphate given by gavage at doses equivalent to 0, 0.0005, 0.005, 0.05, 0.5 and 5 mg nickel/kg body weight/day for seven months).

Groups of 7 female B6C3F1 mice were exposed to 0, 1, 5, and 10 g nickel sulphate/L drinking water. Calculated from the water consumption, these concentrations were equivalent to about 0, 116, 286, and 396 mg nickel sulphate/kg body weight/day or 0, 44, 109, and 151 mg nickel/kg body weight/day. Water consumption, blood and tissues nickel concentration, body and organ weights, histopathology, immune responses, bone marrow cellularity and proliferation as well as cellular enzyme activities were evaluated. Absolute liver weight was significantly decreased in dosed animals and both the absolute and relative thymus weight was significantly and dose-related reduced, even at the lowest dose. The kidney was the major organ of nickel accumulation. The primary toxic effects were expressed in the myeloid system. There were dose-related decreases in bone marrow cellularity, and in granulocyte macrophage and pluripotent stem-cells proliferative responses (Dieter *et al.*, 1988).

Groups of 30 male and 30 female CD rats were administered nickel chloride by gavage at doses of 0, 5, 35, and 100 mg/kg body weight/day for 90 days. In the mid and high dose group, clinical signs of toxicity were seen, body weights and weights of kidney, liver and spleen were reduced and mortality increased. No adverse effects were seen at the dose of 5 mg/kg body weight/day (ABC, 1988).

Groups of 10 Wistar rats of each sex received nickel sulphate in a concentration of 100 mg nickel/L drinking water for 3 and 6 months. The average oral intake was calculated on the basis of drinking water consumption to be 6.9 (males) and 7.6 (females) mg nickel/kg body weight/day. Urinary albumin levels were significantly increased in females exposed for 6 months. The increase observed in males was not significant due to outliers in the controls. No effect was seen on urinary levels of β2-microglobulin or total protein. Kidney weights were significantly increased in the exposed animals (Vyskocil *et al*., 1994).

Groups of 8 male Sprague Dawley rats were given nickel sulphate hexahydrate in drinking water in concentrations of 0, 0.02, 0.05, and 0.1%, i.e. 0, 44.7, 111.75, and 223.5 mg nickel/L for 13 weeks. At the highest dose, final body weight, plasma total proteins, albumin, globulins, glutamic pyruvic transaminase activity and the urine volume were significantly decreased and the lymphocyte subpopulations (T and B cells) suppressed. At the lower dose levels, T and B cells were induced. No gross or microscopic changes were seen in any of the various tissues examined. However, the relative liver weights were significantly decreased in the mid and high dose groups and the relative spleen weights significantly increased in all treated groups as well as the relative kidney weights at both the lowest and highest dose and the relative lung weight at the highest dose. Alkaline phosphatase activity in the bronchoalveolar lavage fluid was significantly decreased at any dose level, indicating a significant decrease in the activity of type II cells in the alveolar space and some early damage to the rat lung (Obone *et al*., 1999).

4.3 Chronic toxicity/Carcinogenicity

The carcinogenicity of nickel and nickel compounds has been assessed by several organisations including IARC (1990, 1999), WHO (1991) and EC (2004).

4.3.1 Animal studies

The data on the carcinogenicity of nickel compounds in experimental animals following oral exposure are very limited. The chronic toxicity and carcinogenicity of nickel sulphate by oral route has been studied in rats and dogs (Ambrose *et al.*, 1976). Wistar rats (25 males and 25 females per group) were exposed to 0, 100, 1000 or 2500 mg/kg nickel in feed for 2 years. Growth was significantly depressed at 1000 and 2500 mg/kg; increased heart and decreased liver weights were observed only in females at 1000 and 2500 mg/kg. No neoplasms or other lesions were observed. Beagle dogs (3 males and 3 females per group) were exposed to 0, 100, 1000 or 2500 mg/kg nickel in feed for two years. Growth was significantly depressed and lung lesions were observed at 2500 mg/kg. No neoplasms were observed. Both studies have strong limitations because of the low number of animals (rats and dogs), the high mortality in rats and the limited reporting of the study design and results. The carcinogenicity of nickel acetate has been tested in three drinking water studies with rats and mice (Schroeder *et al.*, 1964 and 1974; Schroeder and Mitchener, 1975), receiving 0 or 5 mg/L nickel as nickel acetate from the time of weaning until death. Histological examinations were limited to the lungs, heart, liver, kidneys and spleen. No increased incidence of neoplasms was observed in either rats or mice. Also these studies are strongly limited in the design and in the reported results.

Overall, the available data are too limited for an evaluation of the carcinogenic potential of nickel compounds in rodents following oral administration. According to information supplied by industry (NiPERA) to EC (2004), a two-year oral carcinogenicity study with nickel sulphate is planned to be ready in 2005. No data regarding the carcinogenicity of nickel chloride, nickel nitrate, nickel carbonate and nickel metal by oral route have been found.

Much attention has been directed towards a series of long-term inhalation NTP studies (1996 a, b, c,). Rats and mice were exposed to aerosols of nickel subsulfide, nickel oxide or nickel hexahydrate for 2 years. It was concluded that there was clear evidence of carcinogenic activity of nickel subsulfide based on increased incidences of tumours in the lung and in the adrenal medulla. Another inhalation study (78 weeks) of nickel subsulfide in rats, also reported an increase in the incidence of lung tumours (Ottolenghi *et al.*, 1974, quoted by IARC, 1990). No carcinogenic activity was seen in mice. With regard to nickel oxide there was some evidence of carcinogenicity in rats based on increased incidences of tumours in the lung and in the adrenal medulla, with equivocal results in female mice. No carcinogenic activity was seen in rats or mice exposed to nickel sulphate. No studies regarding carcinogenicity of nickel chloride, nickel nitrate and nickel carbonate following inhalation exposure or intratracheal instillation in experimental animals have been located. Injection of various nickel compounds in different ways all have caused malignant tumours, usually sarcomas but also other types, at the site of application (IARC, 1990).

Injection of nickel produces distant tumours of the liver in some strains of mice (IARC, 1990). Intraplacental exposure to nickel acetate followed by exposure of the offspring to the promoter barbital in the drinking water produced renal cortical and pelvic tumours (Diwan *et al.*, 1992). Additionally, pituitary tumours (combined adenomas and carcinomas) were

significantly increased in offspring of both sexes given prenatal nickel acetate only. The results of this study indicate that nickel acetate is a transplacental initiator of kidney tumours and a complete transplacental carcinogen of pituitary tumours. A variety of carcinogenicity studies indicate that metallic nickel can produce tumours when given by intratracheal instillation, subcutaneous, intramuscular or intraperitoneal injection in rats and hamsters (IARC, 1990).

According to IARC (1999), there is also sufficient evidence in experimental animals for the carcinogenicity of implants of metallic nickel and for nickel alloy powder containing approximately 66-67% nickel, 13-16% chromium and 7% iron.

Three studies in experimental animals indicate a possible promoting effect of nickel sulphate, when applied locally to the nasopharynx or the oral cavity, or by the feed to pups; however, the indications are rather weak (IARC, 1990; WHO, 1991). In a two-stage carcinogenesis assay, orally administered nickel chloride in drinking water (600 mg/L) for 25 weeks enhanced the renal carcinogenicity of N-ethyl-N-hydroxyethylnitrosamine in rats, but not the hepatocarcinogenicity in rats after initiation with N-nitrosodiethylamine, the gastric carcinogenicity in rats after initiation with N-methyl-N-nitro-N-nitrosoguanidine, the pancreatic carcinogenicity in Syrian golden hamsters following initiation with N-nitroso-bis (2-oxopropyl)-amine, or the skin carcinogenesis in mice initiated with 7, 12-dimethylbenzanthracene (IARC, 1990; WHO, 1991). Nickel metal weakly enhanced the lung carcinogenicity of 20-methylcholanthrene in rats treated by intratracheal instillation (IARC, 1990). A two-stage carcinogenesis study was carried out in which nickel acetate tetrahydrate (single i.p. injection of 5.3 mg/kg body weight) was tested as a tumour initiator in male rats using sodium barbital (500 mg/L in drinking water) as the promoter (Kasprzak *et al*., 1990). Increased incidences of renal cortical adenomas and combined adenomas and adenocarcinomas were observed.

In conclusion, the available data indicate that nickel sulphate, nickel chloride and nickel metal may have a promoting effect in combination with selected initiators. There is also some evidence, again limited, that soluble nickel compounds may act as promoters also by the oral route.

4.3.2. Human data

Several cohort studies of workers exposed by inhalation to various nickel compounds showed an increased risk of lung and nasal cancer (IARC, 1990). Although the precise compound responsible of the carcinogenic effects in humans was not always clear, studies indicated that nickel sulphate and combinations of nickel sulphides and oxides encountered in the nickel refining industry were responsible for cancer in humans. An additional study had shown that exposure of nickel refinery workers to soluble nickel compounds alone or in combination with other forms of nickel caused significant excess risks for lung and nasal cancer and that smoking and nickel exposure had a multiplicative effect (Andersen *et al*., 1996). Nickel exposure in mild-steel welders has been associated with tumours (carcinomas) of the trachea, bronchus and lung in some cases (Simonato, 1991), although subjects were exposed also to chromium, which complicated the results.

4.3.3 Overall conclusion

IARC (1990) made an overall evaluation of nickel compounds as a group (Group 1: Human carcinogens), based on sufficient evidence of epidemiological information, sufficient evidence in experimental animals and on indications from mechanistic and animal studies that the event responsible for inducing cancer is generation of ionic nickel at target sites.

Recently, the European Commission Working Group of Specialized Experts in the fields of carcinogenicity and mutagenicity, has concluded that nickel sulphate, nickel chloride, nickel nitrate and nickel carbonate, should be considered as human carcinogens by inhalation (Carc. Cat.1, with the risk phrase R49 "May cause cancer by inhalation") (EC, 2004).

The experimental evidence for carcinogenicity of nickel compounds or metallic nickel following oral exposure is lacking; however, the data presently available are very limited. A long-term study with nickel sulphate by oral route, currently being carried out, will provide additional information on which to evaluate this effect. There is also some evidence, although again limited, that soluble nickel compounds may act as promoters by oral route.

4.4 Genotoxicity

The genotoxicity of nickel and nickel compounds has been reviewed by several organisations including ECETOC (1989), IARC (1990), WHO (1991), ATSDR (1997) and EC (2004).

4.4.1 In vitro studies

4.4.1.1 Gene mutation

Most of the presently available data comes from studies in bacteria with nickel chloride. In general, the nickel compounds tested gave negative results in *Salmonella enterica var.* Typhimurium (nickel chloride, nickel sulphate and nickel nitrate) and *Escherichia coli* (nickel chloride and nickel sulphate). Only a fluctuation test gave a weak positive result with nickel chloride in *Salmonella enterica var.* Typhimurium. The overall evidence indicates that nickel compounds are not mutagenic in bacteria. Both nickel chloride and nickel sulphate have been tested in gene mutation tests with different mammalian cells, many of which with weakly positive results (e.g. in mouse lymphoma and V79 cells). In at least some of these assays, the positive results were likely due to genetic events other than gene mutations (e.g. chromosomal aberrations and DNA methylation). For instance, it has been shown that the increase in mutation frequency at the *gpt* gene of V79 cells (Christie *et al.*, 1992) were due to changes in DNA methylation (Klein, 1994). DNA methylation seems to be related to the inhibition of tumour suppressor genes (Costa and Klein, 1999).

4.4.1.2 Chromosomal effects

Chromosomal aberrations (CA) have been extensively studied with nickel chloride and nickel sulphate in cultured mammalian cells (IARC, 1990; WHO, 1991). Positive results, although weak, were seen in almost all studies in the range of 0.59-59 mg nickel/L. A weak (1.5 to 2 fold) increase in sister chromatide exchange (SCE) was also detected at concentrations of 14 and 19 mg/L. Positive results were also seen with nickel carbonate (CA and SCE in CHO cells). Induction of chromosomal aberrations were observed also in cultured human lymphocytes with Ni_3S_2 (7.3-73 mg/L), $NiCO_3$ (0.59-59 mg/L) and $NiSO_4$ (1.1 mg/L). Most

aberrations were gaps rather than breaks or fragments. Disturbance of spindle function was also seen in rat embryo cells (nickel chloride) and human peripheral lymphocytes (nickel sulphate), with also weak positive results in the micronucleus test (kinetochore stained) in human diploid fibroblasts, suggesting that numerical chromosome changes (e.g. aneuploidies) might occur.

4.4.1.3 DNA damage and repair

Most data come from studies with nickel chloride and nickel sulphate (IARC, 1990; WHO, 1991). Both soluble salts induced mitotic gene conversion in yeast, DNA single-strand breaks (SSB) and DNA-protein cross-links (DPC) in cultured mammalian cells. The formation of DPC may involve non-covalent association of DNA and chromatin proteins. SSB are repaired quickly while DPC appear to persist. Nickel ion binds to chromatin more strongly than to DNA. Nickel sulphate induced inhibition of DNA synthesis/repair.

4.4.1.4 Cell transformation

Nickel sulphate and nickel chloride have been shown to induce cell transformation in Syrian hamster cells (SHE), BALB 3T3 and C3H10T1/2 mouse cells (IARC, 1990; WHO, 1991).

Soluble nickel (Ni^{2+}) can act as both an initiator and promoter in SHE cells, although it appears to be a more potent promoter than initiator. Nickel chloride (1 mg Ni/L) produced a ten-fold increase in morphological transformation in BALB 3T3 mouse cells. Nickel subsulphide (0.1-7 mg/L), nickel sulphide (0.3-3 mg/L) and nickel oxide (3-23 mg/L) caused dose-dependent increases in the frequency of transformation in C3H10T1/2 mouse cells, while nickel sulphate and nickel chloride (0.03-6 mg/L) were negative. Several studies have measured the transforming potential (as anchorage independance) in human foreskin cells showing positive effects.

Transformation assays do not directly measure genotoxicity *per se*, but it is generally believed that cell transformation involves some form of genotoxicity, including alterations in DNA sequence and expression.

4.4.2 In vivo studies

4.4.2.1 Gene mutations

Weakly positive effects have been seen in one study in Drosophila (Wing spot mutation) with nickel chloride (Ogawa *et al*., 1994).

No significant increase in mutation frequency was found in the nasal mucosa or lung cells of transgenic LacZCD2F1 mice or lacI F344 rats following *in vivo* inhalation exposure for two hours to nickel subsulphide (Ni_3S_2) at dose levels close to the MTD (Mayer *et al.*, 1998). The results do not support any conclusion regarding the ability of this compound to induce gene mutations *in vivo*, due to the short exposure time, as this model needs 4-6 weeks of exposure for maximal expression of mutations.

4.4.2.2 Chromosomal effects

Most of the studies were carried out with nickel sulphate, chloride and nitrate. Chromosomal aberrations were induced at high doses in bone marrow cells of mice, rats and hamster via oral, intratracheal or intraperitoneal administration (IARC, 1990; IPCS 1991). No increases in SCE were found. The data from micronucleus are conflicting, with negative results (Deknudt and Leonard, 1982; Covance, 2003) and positive results in Indian studies by i.p. (Dhir *et al*., 1991) or by oral gavage (Sharma *et al*., 1987; Sobti and Gill, 1989). Nickel metal was positive in rat bone marrow cells by intratracheal administration (Zhong *et al*., 1990). No significant increase of dominant lethal mutations were reported (Deknudt and Leonard, 1982; Saichenko, 1985). A significant increase in sperm abnormalities was shown by Sobti and Gill (1989).

4.4.2.3 DNA damage and repair

There is evidence that both soluble and insoluble nickel compounds can produce DNA single strand breaks and DNA-protein cross-links *in vivo* in rat liver cells (IARC, 1990). An inhalation study in mice and in rats by Benson *et al*. (2002) has shown DNA single strand breaks in the comet assay after high doses of nickel sulphate and nickel subsulfide. There was no indication of oxidative damage, although inflammation was evident. The DNA damage therefore most likely was related to inflammation and/or apoptosis. Nickel subsulfide but not nickel sulphate was able to induce cell proliferation.

4.4.2.4 Human data

The frequency of chromosomal gaps was significantly increased (3-5-fold) in peripheral T-cells of workers exposed to nickel (Boysen *et al*., 1980; Waksvik and Boysen, 1982; Waksvik *et al*., 1981 a and b). By contrast, no significant increase in the frequency of chromosomal breaks or SCE was found. Cytogenetic studies conducted in retired nickel workers 4-15 years after employment revealed increases both in gaps (1.4-fold) and breaks (8-fold) in peripheral T-cells. No difference in the frequency of SCE was observed (Waksvik *et al*., 1984 a and b). In another bio-monitoring study CAs were measured in peripheral lymphocytes of workers occupationally exposed to oxidic nickel and nickel sulphate in a Czech Republic chemical plant. A significant although small increase (1.6-fold) in the mean value of CAs (gaps, chromatid and chromosome breaks) was found in the combined exposed group compared to the control (Senft *et al*., 1992). Possible confounding factors were not discussed. A cytogenetic study in peripheral lymphocytes of workers occupationally exposed to nickel carbonyl did not reveal significant increases of chromosome breaks or gaps (Decheng *et al*., 1987).

4.4.3 Mechanisms of genotoxicity

The mechanism of the genetic activity of nickel compounds is not clearly defined. Results of *in vitro* and *in vivo* studies have shown that nickel compounds produce DNA single-strand breaks either directly or indirectly. The DNA breaks are the most logic candidates for the initial DNA lesions responsible of the various effects at chromosome level. However, the mechanisms by which DNA breaks are induced are not clear. One hypothesis involves the generation of oxygen free radicals in a process analogous to the Fenton reaction. However, oxidative damage by reactive oxygen species is unlikely to play a major role, due to the observed genetic profile of nickel compounds, with negative results in tests for *hprt* mutations and for oxidative damage of DNA. Inhibition of DNA synthesis seems to be more likely,

which is consistent with the negativity in bacteria. Another possible mechanism may involve inihibition of DNA repair. Impaired repair function has been seen *in vitro* at low non-cytotoxic nickel concentrations (Hartwig and Schwerdtle, 2002). Clearly, more research into the mechanisms of nickel mutagenesis and carcinogenesis are needed.

4.4.4 Comment

There is considerable evidence for the *in vitro* genotoxicity of soluble nickel compounds; the database for nickel carbonate and metallic nickel is much more limited or inadequate. Positive effects are generally seen in studies of chromosomal effects (CA and SCE), DNA damage and repair (SSB and DPC) and cell transformation.

The ability of nickel compounds to induce gene mutations is less clear. The weight of evidence suggests that nickel compounds are unable to efficiently induce point mutations. The few positive results reported for gene locus mutations (*hprt* gene) consist of small effects at high, toxic doses and where the mutations were not characterized at a molecular level. These results are likely to be due to other genetic events than points mutations (frame-shift or base substitution type mutations), e.g. chromosomal aberrations and DNA methylation.

Interpretation of the results of *in vivo* studies is more complicated. The *in vivo* clastogenicity of nickel chloride is the more convincing; however, when taken together all the data presently available for the three soluble compounds (nickel chloride, sulphate and nitrate), there is evidence of *in vivo* genotoxicity at chromosome level in somatic cells, although this is manifested at high, toxic doses. There is also supportive evidence from studies on workers exposed by inhalation to nickel compounds, showing increased frequencies of chromosomal gaps or aberrations.

Evidence for any possible effect on germ cells is particularly limited. There is evidence that the nickel ion reaches the testis in rodents after i.p. administration, but there are few data on possible effects.

Recently (EC, 2004) the European Commission Working Group of Specialized Experts in the fields of carcinogenicity and mutagenicity has proposed the following classification of nickel sulphate, nickel chloride and nickel nitrate: Mutag. Cat. 3, with the risk phrase R68 “Possible risk of irreversible effect”. This conclusion was based on evidence of *in vivo* genotoxicity in somatic cells, after systemic exposure. Hence, the possibility that germ cells are affected could not be excluded. However further testing of effects on germ cells was not considered practicable. There was insufficient evidence for classification of the mutagenicity of nickel carbonate.

4.5 Reproductive and developmental toxicity

In a 3 generation study, groups of Wistar rats were fed diets containing 0, 250, 500 and 1000 mg/kg nickel as nickel sulphate hexahydrate, equivalent to about 0, 12.5, 25 or 50 mg nickel/kg body weight/day. In all groups, 20 rats of each sex were mated. In the first generation, the number of stillborn rats was increased at all dietary levels. The number of siblings per litter and siblings weaned decreased with increasing doses. At the highest dose, body weights of weanlings were markedly reduced. No adverse effects were noted on fertility, gestation, viability and lactation indices. Teratogenic effects were not observed (Ambrose *et al.*, 1976).

In a 2 generation study, nickel chloride was administered in drinking water to groups of 30 CD rats of each sex at dose levels of 0, 50, 250 and 500 mg/L, equivalent to 0, 7.3, 30.8 and 51.6 mg/kg body weight/day, for 90 days before breeding. At the highest dose, there was a significant decrease in the maternal body weight, along with absolute and relative liver weights. In the F_{1a} generation, at this dose the number of live pups/litter was significantly decreased, pup mortality significantly increased and average pups body weight significantly decreased. In the F_{1b} litters, increased pup mortality and decreased live litter size was also observed in the lower dose groups. These effects are questionable, however, because the room temperature was higher than normal at certain times along with lower levels of humidity (RTI, 1987).

Groups of 34 female Long-Evans rats received nickel chloride in drinking water for 11 weeks prior to breeding and during two successive gestation and lactation periods at doses of 0, 10, 50, and 250 mg nickel/L equivalent to 1.33, 6.8 or 31.8 mg nickel/kg body weight/day. At the highest dose in the first generation and at all doses in the second generation, a dose-related increase in pups born dead or dying shortly after birth was observed. Body weight gain was reduced in dams of the mid and top dose groups (Smith *et al*., 1993).

4.6 Human data

Twenty of 32 electroplating workers, who inadvertently drank water contaminated with nickel sulphate and chloride, developed symptoms, such as nausea, vomiting, diarrhoea, giddiness, lassitude, headache, cough and shortness of breath. Laboratory tests showed elevated levels of blood reticulocytes, urine albumin, and serum bilirubin. The nickel doses that caused these symptoms were estimated to be in the range of 7.1-35.7 mg/kg body weight (Sunderman *et al*., 1988).

Nickel salts are potent skin sensitisers in humans, causing allergic contact dermatitis. Nickel ions bind to cellular and matrix proteins of the skin and induce a cellular immune response (type IV hypersensitivity reaction) (Büdinger et *al.*, 2000). The prevalence of nickel sensitivity in the population is about 8-14.5% for adult women and about 1% for men (WHO, 1996). In sensitised individuals, not only dermal exposure, but also oral intake of low doses can provoke eczema.

In a number of oral challenge studies, single oral doses of a few mg nickel provoked dermal reactions in nickel-sensitised subjects (Christensen and Möller, 1975; Kaaber *et al*., 1978; Gawkrodger *et al*., 1986; Menne and Maibach, 1991). The lowest oral doses, given to nickel sensitive subjects and reported to exacerbate hand eczema, were 0.49 mg/day in a high nickel diet (Nielsen *et al*., 1990), equivalent to about 8 μg nickel/kg body weight/day, and 12 μg/kg body weight/day given in drinking water on an empty stomach (Nielsen *et al*., 1999).

CONCLUSIONS AND RECOMMENDATIONS

1. DOSE RESPONSE ASSESSMENT AND DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

In studies on subchronic toxicity, the main targets for the toxicity of orally ingested nickel salts are kidneys, spleen, lungs, and the myeloid system. In addition, perinatal mortality has

been reported to increase in rats, even at the lowest administered dose of 1.3 mg nickel/kg body weight/day. The available studies do not allow the establishment of a NOAEL.

There is evidence that nickel salts are carcinogenic in rodents and humans by inhalation. The evidence for carcinogenicity following oral exposure is lacking, however the data presently available are very limited. The Panel notes that a long-term study with nickel sulphate in rats by the oral route, which will improve the presently limited data-base, is on-going.

The genotoxicity of nickel salts, observed at chromosome level at high, toxic doses is likely due to indirect mechanisms.

It is not possible to derive a threshold for provoking dermal reactions in nickel-sensitised subjects. Although only dermal exposure to nickel can lead to sensitisation, oral doses of nickel have been shown to exacerbate hand eczema in nickel-sensitised individuals. In some studies, as little as 8 and 12 μg nickel/kg body weight provoked such reactions.

In the absence of adequate dose-response data for these effects, it is not possible to establish a tolerable upper intake level.

2. RISK CHARACTERIZATION

Nickel has not been demonstrated to be essential for humans.

Estimates of nickel intake from the average diet range from 80 to 150 μg/person/day, but may reach 900 μg/person/day or more, when large amounts of food items with high nickel contents are consumed. An intake of 900 μg nickel/person/day, equivalent to 15 μg/kg body weight/day, would be 90-fold lower than the lowest dose of 1.33 mg nickel/kg body weight/day reported to increase the perinatal mortality of rats. In the worst case, however, the first-run drinking water, which remained in the tap overnight, would be consumed and the release of nickel from kitchen utensils would cause an additional alimentary exposure. In that case, the margin between the dietary intake and toxic doses would be considerably lower.

In the group of nickel-sensitised persons, the margin of exposure is even lower. An intake of 150 μg nickel/person/day from the average diet, equivalent to 2.5 μg/kg body weight/day in a 60 kg adult, is about one third of the lowest reported dose of 8 μg nickel/kg body weight (490 μg/day) able to cause flare-ups of hand eczema in sensitised subjects. Consumption of food with high nickel content and additional exposure from first-run drinking water and kitchen utensils could result in an intake higher than the critical dose.

Any additional nickel intake from supplements would further increase the risk. In this context, the Panel draws attention to the high prevalence of nickel sensitisation in the population and to the fact, that many individuals may not be aware that they are sensitised.

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