

***Recommendation from the Scientific Committee on
Occupational Exposure Limits for
Nickel and Nickel Compounds***

| | | |
|---------------------------|---|---|
| 8 hour TWA | : | 0.01 mg/m ³ (inhalable fraction) |
| STEL (15 min) | : | -- |
| Additional classification | : | sensitiser |
| BLV | : | see biomonitoring section |
| SCOEL carcinogen group | : | C (carcinogen with a practical threshold) |

1. Substance Identity and Properties:

The document deals with nickel and its soluble and insoluble compounds. The following table presents examples of these compounds.

| Substance, synonyms | Molecular formula | CAS Number | EINECS Number | Molecular weight | Solubility in water (g/100 ml) [temperature] | Classification |
|---|--------------------------------|---------------|------------------|---------------------|--|--|
| Nickel metal | Ni | 7440-02-0 | 231-111-4 | 58.71 | poorly soluble | Carc. Cat. 3; R40 T; R48/23 R43 |
| Nickel monoxide, nickel(II) oxide | NiO | 1313-99-1 | 215-215-7 | 74.69 | poorly soluble | Carc. Cat.1; R49, T; R48/23 R43 |
| Nickel dioxide, nickel(IV) oxide | NiO ₂ | 12035-36-8 | 234-823-3 | 90.69 | poorly soluble | Carc.Cat.1; R49, T; R48/23 R43 |
| Dinickel trioxide, nickel trioxide, nickel(III) oxide | Ni ₂ O ₃ | 1314-06-3 | 215-217-8 | 165.38 | poorly soluble | Carc.Cat.1; R49, T; R48/23 R43 |
| Nickel hydroxide | Ni(OH) ₂ | 12054-48-7 | 235-008-5 | 92.72 | 0.013 | Carc.Cat.1; R49, Repr. Cat. 2; R61 Muta. Cat3; R68 T; R48/23 Xn;R20/22, Xi; R38 |

SCOEL/SUM/85 Nickel and Nickel Compounds

| Substance, synonyms | Molecular formula | CAS Number | EINECS Number | Molecular weight | Solubility in water (g/100 ml) [temperature] | Classification |
|---------------------|---|------------|---------------|------------------|--|--|
| Nickel carbonate | NiCO ₃ | 3333-67-3 | 222-068-2 | 118.70 | 0.0093 [25°C] | R42/43 Carc.Cat.1; R49, Repr. Cat. 2; R61 Muta. Cat3; R68 T; R48/23 Xn;R20/22, Xi; R38 R42/43 |
| Nickel sulfide | NiS | 16812-54-7 | 240-841-2 | 90.77 | 0.00036 [18°C] | Carc.Cat.1; R49, Muta. Cat3; R68 T; R48/23 R43 |
| Nickel subsulfide | Ni ₃ S ₂ | 12035-72-2 | 234-829-6 | 240.26 | poorly soluble | Carc.Cat.1; R49, Muta. Cat3; R68 T; R48/23 R43 |
| Nickel chloride | NiCl ₂ | 7718-54-9 | 231-743-0 | 129.61 | 254 [20°C] | Carc.Cat.1; R49, Repr. Cat. 2; R61 Muta. Cat3; R68 T; R3/25-48/23 Xi; R38-41 R42/43 |
| Nickel nitrate | Ni(NO ₃) ₂ | 13138-45-9 | 236-068-5 | 182.72 | 238.5 [0°C] | Carc.Cat.1; R49, Repr. Cat. 2; R61 Muta. Cat3; R68 T; R48/23 Xn;R20/22, Xi; R38-41 R42/43 |
| Nickel sulfate | NiSO ₄ | 7786-81-4 | 232-104-9 | 154.77 | 65.5 [0°C] | Carc.Cat.1; R49, Repr. Cat. 2; R61 Muta. Cat3; R68 T; R48/23 Xn;R20/22, Xi; R38 R42/43 |
| Nickel acetate | Ni(CH ₃ CO ₂) ₂ | 373-02-4 | 206-761-7 | 176.80 | 17 [20°C] | Carc.Cat.1; R49, Repr. Cat. 2; R61 Muta. Cat3; R68 T; R48/23 Xn;R20/22, R42/43 |

This Summary document is based on documentations from IARC (1990), ICPS (1991) and the German MAK Commission (Greim 2006) as well as on reviews (Denkhaus and Salnikow 2002, Haber *et al.* 2000) and the EU RAR (EU RAR 2008 a-f).

The average nickel intake by non-occupationally exposed adults varies from 0.1 mg to 0.9 mg per day with an average value of 0.2 mg per day. Main exposure results from food (150 – 250 µg per day), drinking water (up to 20 µg per day) and cigarettes (4 – 8 µg per 20 cigarettes). Nickel uptake from inhalative exposure to ambient air is rather low (about 0.04 µg in cities). Nevertheless, the bioavailability from oral intake is low (about 2 %), while absorption from ambient air or cigarettes is 100 or 50 %, respectively (summarized in IPCS, 1991 and EU RAR, 2008 b).

Occurrence/ use and occupational exposure

Nickel is a hard, silvery-white metal. It is very widely used in the heavy industries (mining, milling, foundries, refining) and in the manufacturing industries (production of stainless steel and steel alloys, production of nickel alloys, hot cutting and welding, nickel plating, chemical production and mixing, manufacture of catalysts, manufacture of nickel-cadmium batteries, manufacture of coins, jewellery, pigments, and powders). Nickel species relevant for occupational exposure include metallic nickel, poorly soluble nickel species such as oxides and sulfides as well as water soluble nickel salts.

2 Health significance

2.1 Toxicokinetics

Absorption and distribution

The penetration in the organism, the absorption and the elimination of nickel and its compounds depend on their physical state and largely on the route of exposure. In humans, nickel ions can be taken up via the skin, via the gastrointestinal tract or by inhalation (Grandjean 1984).

Exposure to the poorly soluble oxidic and sulfidic nickel compounds in workplace air **via inhalation** leads to the accumulation of nickel in the lungs (Andersen and Svenes 1989, Angerer *et al.* 1989, IARC 1990, Norseth 1986, Raithel 1987, Sunderman *et al.* 1986, Svenes and Andersen 1998). Readily soluble nickel salts are accumulated to a lesser extent in the lung than poorly soluble oxides and sulfides. For example, in a study in Norway, lower nickel concentrations were found in the lungs of persons exposed mainly to readily soluble nickel salts (50-fold accumulation compared with nonexposed persons) than in those of persons exposed to poorly soluble nickel compounds (400- to 500-fold accumulation) (Svenes and Andersen 1998). A mathematical modelling of the deposition and clearance of inhaled nickel compounds on the basis of data from animal inhalation studies with nickel monoxide, nickel subsulfide and nickel sulfate also showed that the lung retention times in rats and humans are substantially longer for the poorly soluble compounds nickel monoxide and nickel subsulfide than for the readily soluble nickel sulfate (Hsieh *et al.* 1999 a, b). A retention time comparable to poorly soluble nickel compounds was found for metallic nickel in an inhalation study with Wistar rats (NIPERA, 2008). Concerning the **bioavailability of different nickel species** after inhalation, all compounds are bioavailable, even though to a different extent. In rats, about 98 % or 6 % were bioavailable after inhalation of water soluble nickel or metallic nickel, respectively. Similarly, in humans, increased nickel concentrations were detected in the blood and urine of workers exposed to both readily soluble and poorly soluble nickel compounds including metallic nickel,

indicating the bioavailability of all nickel species (Angerer *et al.* 1989, IARC 1990, Raithel 1987).

On the cellular level, nickel ions are relatively slowly absorbed via ion channels of cell membranes (Costa 1996), while poorly soluble nickel compounds and metallic nickel can be taken up by mammalian cells by phagocytosis, followed by the release of nickel ions due to gradual dissolution of nickel particles in the lysosomes (Costa and Mollenhauer 1980). When comparing the uptake and distribution in cultured A549 cells, both water soluble nickel chloride and particulate nickel oxide increased the nickel content in the cytoplasm and the nucleus, with a higher fraction of nickel reaching the nucleus in case of nickel oxide (Schwerdtle and Hartwig, 2006). In rats, poorly soluble nickel compounds and metallic nickel deposited in the lungs by inhalation were phagocytosed by alveolar macrophages (Johansson *et al.* 1980).

The bioavailability of nickel compounds by the **oral route** depends strongly on the nickel species. After oral administration to rats, the readily soluble nickel salts were absorbed to the greatest extent, whereas nickel oxides and nickel metal were absorbed only to a slight extent. Thus, absorption was found to be 34% for nickel nitrate, 11% for nickel sulfate, 9.8% for nickel chloride, 0.47% for nickel subsulfide, and 0.01% for nickel oxide (Haber *et al.* 2000). Nickel ingested accumulated particularly in the kidneys (Ishimatsu *et al.* 1995).

The **skin** constitutes a more or less impenetrable barrier to nickel compounds. Certain water-soluble compounds can cross this barrier, in particular nickel salts and also nickel tetracarbonyl which, because of its liposolubility, readily passes through biological membranes

2.2 Acute Toxicity

Nickel at high doses and in certain forms is toxic to both man and animals. For example, the oral LD₅₀ of nickel acetate was 350 mg/kg bw in rats and 420 mg/kg bw in mice. In case of nickel chloride intraperitoneal LD₅₀ values in rats were 11 and 48 mg/kg bw in mice. Only a small number of reports of acute nickel toxicity caused by inorganic nickel intake are described in the literature. The most severe poisoning is caused by exposure to Ni(CO)₄ (Denkhaus and Salnikow 2002).

2.3 Irritation

Metallic nickel is not a skin or an eye irritant. In rabbits, nickel sulphate was not a skin irritant. However, human data indicate that nickel sulphate in concentrations above 20% can induce skin irritation. Nickel sulphate is not an eye irritant in experimental animals. There are no data on irritation and corrosivity for nickel hydroxycarbonate and nickel chloride (EU RAR 2008 a, c, d).

2.4 Sensitization

Effects in humans

Skin

Nickel is the most commonly diagnosed cause of allergic contact dermatitis worldwide. In the vast majority of cases the cause can be determined as non-workplace-related contact to nickel-containing jewellery or commodities (Bruynzeel et al. 2005; Goon und Goh 2005; Schnuch et al. 2004; Uter et al. 2005). It is still possible that an exposure to nickel salts in a work situation (for instance in the electroplating industry) might evoke contact sensitization too (Li et al. 2003; Lidén 1994, 1998; Meding et al. 1994; Uter et al. 2003). In 2004 11 643 patients were patch tested in 11 European countries within the European Surveillance System of Contact Allergies (ESSCA). The proportion of patients testing positive for nickel sulphate was 20% and the proportion among patients with an occupational dermatitis was around 27% (The ESSCA Writing Group 2008).

Respiratory tract

Published results indicate that nickel can additionally induce an IgE-mediated respiratory sensitization (Dolovich et al. 1984; Estlander et al 1993; Fernández-Nieto et al. 2006; Kusaka et al. 1991; Nieboer et al. 1984). There are actually few case reports suggesting evidence for specific IgE, positive skin tests and positive provocation tests with nickel sulfate in exposed persons, and pointing to a workplace related asthmatic pathology (Bright et al. 1997; Cirila et al. 1982). The incidence of occupational asthma among stainless steel welders in Finland was 0.9-2 per 1000 workers each year (Hannu et al 2007).

Since nickel ions can be released from nickel metals or nickel compounds of low solubility, the above applies to metals, compounds, and nickel alloys, from which nickel is biologically available (Hartwig 2008).

Effects in experimental animals

Until 1989 more than 25 different procedures were described for the determination of the sensitizing potential of nickel in animal studies (Wahlberg 1989). Nine of 14 and 12 of 14 Dunkin-Hartley guinea pigs reacted positively after open epicutaneous application of nickel sulfate in lanolin at concentrations of 1% and 3% respectively. When hydroxypropylcellulose was used as a vehicle, 5 of 12 showed a positive reaction to 0.3% nickel sulfate, 7 of 12 to 1% and 4 of 12 to 3% nickel sulfate. Irritation to the skin was induced more often by lanolin than by hydroxypropylcellulose (Nielsen *et al.* 1992).

2.5 Repeated dose toxicity

The target organ for non-cancer effects of inhalation exposure to nickel is the respiratory tract, with effects seen in both the lungs and the nose. A variety of inflammatory lesions (e.g., chronic inflammation, interstitial infiltrates) have been identified in the lungs of rats and mice following subchronic and chronic inhalation exposures. Atrophy of the olfactory epithelium was also observed. The histopathology data are supported by biochemical evidence of lung damage, based on increased enzyme levels in BAL fluid (Haber *et al.* 2000).

In a study performed by NTP (1996 c), male and female rats were exposed to nickel sulfate hexahydrate aerosol at concentrations of 0, 0.12, 0.25, or 0.5 mg compound/m³ (0, 0.027, 0.056, or 0.11 mg Ni/m³) for 6 h/day, 5 days/week for 2 years. At the 2-year sacrifice, nonneoplastic inflammatory lesions of the lung were observed at exposure concentrations ≥ 0.056 mg Ni/m³ in males and females, with no elevation over control incidence at the low concentration (see Table 1). NTP reported "alveolar macrophage hyperplasia" in all males and four of five females at 0.056 mg Ni/m³ in the 7-month evaluation. In the 15-month evaluation, the incidence at this concentration (two of five males, three of five females) was not statistically different from controls (0 of five males, one of five females). The incidence of "alveolar macrophage hyperplasia" at the low concentration (0.027 mg Ni/m³) was not statistically significant following exposure for 15 months or 2 years. A definitive conclusion regarding the adversity of the endpoint is not possible. The macrophage accumulation may be a secondary response to tissue damage and/or may contribute to inflammation, which could progress to fibrosis. However, such a progression was not clearly supported or refuted by the results of the chronic bioassay. **The no observed adverse effect level (NOAEL) for lung effects in rats in the chronic study is taken as 0.027 mg Ni/m³.** The NOAEL for atrophy of the olfactory epithelium was 0.056 mg Ni/m³ (Haber *et al.* 2000).

Two further chronic inhalation studies were performed by NTP. In one study, male and female rats were exposed to nickel subsulfide at concentrations of 0, 0.15, or 1 mg compound/m³ (0, 0.11, 0.73 mg Ni/m³) for 6 h/day, 5 days/week for 2 years. Even at the lower concentration, fibrosis, inflammation and alveolar hyperplasia in the lungs were observed with a high incidence in practically all animals. Bronchiolar hyperplasia and cellular infiltration at the interstitium were observed with a lower, but still highly significant incidence (see Table 1; NTP 1996 b).

In the other NTP-study, male and female rats were exposed to nickel oxide at concentrations of 0, 0.62, 1.25, or 2.5 mg compound/m³ (0, 0.5, 1.0, 2.0 mg Ni/m³). At all three concentrations chronic inflammation and alveolar pigmentation was observed at high incidence in all animals (see Table 1; NTP 1996 a).

In a recent inhalation study with 24 month whole-body exposure of male and female Wistar rats to 0, 0.1, 0.4, or 1.0 mg Ni/m³ (nickel metal powder) for 6 h/day, 5 d/week, high mortality was observed in the highest dose group. Mortality was also increased at 0.4 mg Ni/m³, most pronounced in female rats. Mean body weights in the 0.4 mg/m³ group males and females were 27% and 18% lower than controls, respectively. In the 0.1 mg/m³ exposure group, significantly reduced body weight (11%) was noted only for the males. Respiratory tract lesions (proteinosis, alveolar histiocytosis, chronic inflammation, bronchiolar-alveolar hyperplasia) and histiocyte

infiltration in the bronchial lymph node were noted in male and female animals of the 0.1 and 0.4 mg/m³ groups (Oller et al. 2008). No NOAEL could be derived. The LOAEL of this study is 0.1 mg Ni/m³.

Table 1a Selected incidences of lung lesions in rats in 2-year inhalation studies with nickel compounds (NTP 1996 a, b, c)

| Lung lesions | | Concentration (mg Ni/m ³) | | | | | | | | | | | |
|--|---|---------------------------------------|-------|----------------|----------------|-------------------|----------------|----------------|--------------|----------------|----------------|----------------|--|
| | | Nickel sulfate hexahydrate | | | | Nickel subsulfide | | | Nickel oxide | | | | |
| | | 0 | 0.03 | 0.06 | 0.11 | 0 | 0.11 | 0.73 | 0 | 0.5 | 1.0 | 2.0 | |
| Fibrosis ^a | ♂ | 3/54 | 6/53 | 35/53** | 43/53** | 2/53 | 48/53** | 40/53** | | | | | |
| | ♀ | 8/52 | 7/53 | 45/53** | 49/54** | 0/53 | 50/53** | 44/53** | | | | | |
| Inflammation, chronic | ♂ | 14/54 | 11/53 | 42/53** | 46/53** | 9/53 | 53/53** | 51/53** | 28/54 | 53/53** | 53/53** | 52/52** | |
| | ♀ | 14/52 | 13/53 | 49/53** | 52/54** | 7/53 | 51/53** | 51/53** | 18/53 | 52/53** | 53/53** | 54/54** | |
| Alveolus pigmentation | ♂ | | | | | | | | 1/54 | 53/53** | 53/53** | 52/52** | |
| | ♀ | | | | | | | | 0/53 | 52/53** | 53/53** | 54/54** | |
| Alveolus hyperplasia macrophage | ♂ | | | | | 9/53 | 48/53** | 52/53** | | | | | |
| | ♀ | | | | | 8/53 | 51/53** | 52/53** | | | | | |
| Macrophage hyperplasia | ♂ | 7/54 | 9/53 | 35/53** | 48/53** | | | | | | | | |
| | ♀ | 9/52 | 10/53 | 32/53** | 45/54** | | | | | | | | |
| Alveolar proteinosis | ♂ | 0/54 | 0/53 | 12/53** | 41/53** | 1/53 | 36/53** | 51/53** | | | | | |
| | ♀ | 1/52 | 0/53 | 22/53** | 49/54** | 2/53 | 49/53** | 53/53** | | | | | |
| Bronchus hyperplasia, lymphoid | ♂ | | | | | 0/53 | 10/53** | 14/53** | | | | | |
| | ♀ | | | | | 0/53 | 15/53** | 18/53** | | | | | |
| Interstitialium, infiltration cellular | ♂ | | | | | 17/53 | 31/53* | 39/53** | | | | | |
| | ♀ | | | | | 28/53 | 36/53 | 43/53** | | | | | |

^a In case of nickel oxide, varying degrees of parenchymal and subpleural fibrosis were present within the inflammatory foci.

* p<0.01 (Fisher's exact test), ** p<0.001 (Fisher's exact test)

Table 1b Selected non-neoplastic histopathological lung lesions in rats in 2-year inhalation studies with nickel metal (Oller et al. 2008)

| Selected lesions | Males | | | Females | | |
|--|---------------------------------------|-----|-----|---------|-----|-----|
| | Concentration (mg Ni/m ³) | | | | | |
| | 0 | 0.1 | 0.4 | 0 | 0.1 | 0.4 |
| Lung^a | | | | | | |
| Proteinosis | | | | | | |
| alveolar | | | | | | |
| Minimal | 0 | 6 | 0 | 8 | 2 | 2 |
| Mild | 0 | 25 | 10 | 0 | 26 | 14 |
| Moderate | 0 | 19 | 15 | 0 | 18 | 16 |
| Serve | 0 | 0 | 25 | 0 | 4 | 22 |
| Histiocytosis | | | | | | |
| alveolar | | | | | | |
| Minimal | 26 | 13 | 8 | 20 | 5 | 20 |
| Mild | 2 | 30 | 19 | 6 | 36 | 20 |
| Moderate | 0 | 7 | 15 | 0 | 9 | 10 |
| Serve | 0 | 0 | 2 | 0 | 0 | 0 |
| Chronic inflammation^b | | | | | | |
| Minimal | 13 | 20 | 8 | 14 | 7 | 16 |
| Mild | 1 | 23 | 11 | 2 | 28 | 6 |
| Moderate | 0 | 1 | 18 | 0 | 10 | 20 |
| Serve | 0 | 0 | 4 | 0 | 0 | 3 |
| Hyperplasia | | | | | | |
| bronchiolar-alveolar | | | | | | |
| Minimal | 1 | 1 | 1 | 0 | 1 | 1 |
| Mild | 1 | 3 | 6 | 0 | 8 | 5 |
| Moderate | 1 | 3 | 5 | 0 | 6 | 2 |
| Serve | 0 | 0 | 4 | 1 | 3 | 1 |
| Bronchial lymph node^c infiltrate | | | | | | |
| histiocyte | | | | | | |
| Minimal | 4 | 8 | 7 | 2 | 11 | 7 |
| Mild | 0 | 12 | 11 | 0 | 13 | 11 |
| Moderate | 0 | 4 | 7 | 0 | 7 | 4 |
| Serve | 0 | 0 | 2 | 0 | 1 | 0 |
| Survival of rats | | | | | | |
| 103 weeks (end of exposure) | | | | | | |

| | | | | | | |
|----------------------------------|----|----|----|----|----|----|
| Number of animals | 41 | 41 | 36 | 30 | 38 | 24 |
| Survival % | 82 | 82 | 72 | 76 | 76 | 48 |
| 130 weeks (scheduled euthanasia) | | | | | | |
| Number of animals | 25 | 18 | 23 | 22 | 19 | 7 |
| Survival % | 50 | 36 | 46 | 44 | 38 | 14 |

^aIncidence based on 50 animals per group, except in Group 3 (0.4 mg Ni/m³) that had 54 females;

^bChronic inflammation includes both chronic and chronic-active inflammation;

^cIncidence based on the following number of animals per group: 34 and 39 for Group

In a chronic mouse study (NTP 1996 c), male and female mice were exposed to 0, 0.25, 0.5, or 1 mg compound/m³ (0, 0.056, 0.11, or 0.22 mg Ni/m³). As in the rats, histologic lesions were confined to the respiratory tract. In females, chronic active inflammation, bronchialization, and alveolar macrophage accumulation were observed at the lowest exposure level (0.056 mg Ni/m³) and higher. The same lesions were observed at ≥ 0.11 mg Ni/m³ in males. Interstitial infiltration and alveolar proteinosis were also observed in females at ≥ 0.11 mg Ni/m³ and in males at 0.22 mg Ni/m³. In the bronchial lymph node, macrophage accumulation occurred in both sexes at ≥ 0.11 mg Ni/m³, and lymphoid hyperplasia was seen in both sexes at the high concentration. Atrophy of the olfactory epithelium was also observed in males at ≥ 0.11 mg Ni/m³ and in females at the high concentration (Haber *et al.* 2000). No NOAEL could be derived from this study.

2.6 Genotoxicity

Effects in humans

Main effects are summarized in Table 2. No increased frequency of chromosomal aberrations in peripheral lymphocytes were found in persons exposed to **metallic nickel** (IARC 1990). There was a higher incidence of metaphases with gaps, but no or only not significantly increased frequencies of sister chromatid exchanges in lymphocytes of persons exposed to **soluble nickel compounds** in electrolytic nickel refining or in nickel plating plants (IARC 1990). A more recent study among workers of an electrolytic nickel refinery in which state-of-the-art protective measures had been taken showed no increased formation of micronuclei in epithelial cells of the buccal mucosa (Kiilunen *et al.* 1997). There was a higher incidence of metaphases with gaps, but no or only not significantly increased frequencies of sister chromatid exchanges in lymphocytes of persons exposed to **sulfidic** and **oxidic nickel compounds** in a nickel smeltery (IARC 1990). Some increase in chromosomal aberrations in lymphocytes was observed in case of NiO exposure at 0.77 mg/m³ (6 exposed individuals); in the same study, workers exposed to soluble nickel (mean exposure 1.3 mg/m³) showed only weak increases in chromosomal aberrations. Taken together, except for one study with 7 exposed individuals (Deng *et al.*, 1988) chromosomal aberrations apart from gaps are restricted to exposure conditions above 0.5 mg/m³. It has to be noted, however, that most studies have been conducted in lymphocytes, since

respiratory epithelial cells as primary targets of nickel carcinogenicity cannot easily be assessed in humans.

Table 2. Genotoxic effect in persons exposed to nickel

| Industry | Exposure | Results | References |
|--|---|--|-----------------------------|
| Nickel smelting (n=9) | 0.1-1.0 mg/m ³ NiO and NiS, 3-33 years exposure, 4.2 µg Ni/l plasma | 11.9% metaphases with gaps (controls 3.7%), no increased SCE frequency | Waksvik and Boysen 1982 |
| Nickel electrolysis (n=10) | 0.1-0.5 mg/m ³ NiCl and NiSO ₄ , 8-31 years exposure, 5.2 µg Ni/l plasma | 18.3% metaphases with gaps (controls 3.7%), no increase in breaks, no increased SCE frequency | Waksvik and Boysen 1982 |
| Nickel smelting and electrolysis (n= 11) | 1 mg Ni/m ³ , >25 years exposed (8 years after retirement) | Increased gaps and breaks | Waksvik <i>et al.</i> 1984 |
| Electroplating with nickel (n=7) | 0.005-0.094 mg Ni/m ³ | 4.3% chromosome aberrations (4 breaks, 3 fragments, 0 exchanges; controls 0.8%), SCE frequency: 7.50±2.19 (controls 6.06±2.30) | Deng <i>et al.</i> 1988 |
| NiO production (n=6) | mean : 0.77 mg/m ³ (range : 0.28 – 1.52) | 9.5% chromosome aberrations as compared to 4.05 % | Senft <i>et al.</i> 1992 |
| NiSO ₄ production (n=15) | mean: 1.3 mg/m ³ (range: 0.28 – 1.52) | 5.2% chromosome aberrations as compared to 4.05 % | Senft <i>et al.</i> 1992 |
| Control group (n=19) | control group later recognised as also exposed to nickel | 4.05% chromosome aberrations (normal value ≤2%) | Senft <i>et al.</i> 1992 |
| Electrolysis (n=25) | 230-800 µg Ni/m ³ workplace air; 0.9-2.4 µg Ni/m ³ behind face mask | no increased number of micronuclei in buccal mucous cells | Kiilunen <i>et al.</i> 1997 |

Effects in experimental systems

In vitro

The genotoxic properties of important nickel compounds *in vitro* are summarized in Table 3.

Metallic nickel caused morphological cell transformation in Syrian hamster embryo (SHE) cells. In contrast, it did not lead to chromosome aberrations in cultured human lymphocytes (IARC 1990). **Nickel chloride** induced DNA breaks, chromosome aberrations (all types), sister chromatid exchanges and, to a slight extent, gene mutations in mammalian cells *in vitro* (IARC 1990). **Crystalline nickel sulfide**, **crystalline nickel subsulfide** and **nickel oxide** caused morphological cell transformation in Syrian hamster embryo cells. Crystalline nickel sulfide and crystalline nickel subsulfide induced DNA breaks, chromosome aberrations (all types), sister chromatid exchanges and, to a slight extent, gene mutations in mammalian cells *in vitro* (IARC 1990). Nickel subsulfide was found to be mutagenic in a transgenic rat embryo fibroblast line (Mayer *et al.* 1998). While mutagenicity is usually weak and restricted to relatively high concentrations, enhancing effects in combination with other genotoxic agents are more pronounced and have been observed at lower concentrations. Thus, nickel ions enhanced the

transformation of hamster embryo cells by benzo[a]pyrene, the mutagenicity of methyl methanesulfonate in *E. coli* as well as the mutagenicity and induction of sister chromatid exchange by UV radiation in hamster cells (IARC 1990). As underlying mechanism, the inhibition of removal of UVC- and benzo[a]pyrene-induced DNA lesions has been demonstrated (IARC, 1990; Schwerdtle et al., 2002).

Table 3. Genotoxic properties of nickel compounds *in vitro*

| Substance | Test system | Result (lowest effective dose in µg Ni/ml) | References | |
|----------------------------|---------------------------------------|---|--------------------|----------------------|
| Nickel metal | peripheral human lymphocytes | no chromosome aberrations | IARC 1990 | |
| | Syrian hamster embryo cells | morphological transformation (20) | | |
| Nickel sulfate | human lymphocytes | increased chromosome aberrations (1.0) and increased SCE (4 studies: 1.4; 0.6; 0.1; 0.6) | IARC 1990 | |
| | human lymphocytes | induction of micronuclei (1.0), increased SCE (5.0) | | Katsifis et al. 1998 |
| | primary human kidney epithelium cells | no increase in DNA breaks immortalisation (5.0) | | |
| Nickel chloride | various bacteria strains | mainly negative mutagenicity tests | IARC 1990 | |
| | CHO cells | DNA breaks and DNA protein cross links (0.45), increased SCE (6.0) induction of DNA repair synthesis (5.9) increased chromosome aberrations (6.0) | | |
| | CHO cells | gaps, breaks, SCE, dicentric chromosomes, fragments | Lin et al. 1991 | |
| | mouse mammary carcinoma cells | increased chromosome aberrations (35.0) | | |
| | mouse mammary carcinoma cells | weakly mutagenic in hprt gene (11.7) | Morita et al. 1991 | |
| | V79 hamster cells | weakly mutagenic in hprt gene (17.7) | | |
| | mouse lymphoma cells | weakly mutagenic in tk gene (10.0) | | |
| Nickel oxide | peripheral lymphocytes | no increased chromosome aberrations | IARC 1990 | |
| | Syrian hamster embryo cells | morphological transformation (14.0) | | |
| | hamster BHK 21 cells | morphological transformation (4.0) | | |
| | human fibroblasts | surface independent growth (3.0) | | |
| Dinickel trioxide | Syrian hamster embryo cells | morphological cell transformation (5.0) | IARC 1990 | |
| Crystalline nickel sulfide | Syrian hamster embryo cells | morphological transformation (6.5) | IARC 1990 | |
| | rat hepatocytes | DNA breaks (114.0) | | |
| | CHO cells | DNA breaks and DNA protein cross links (6.5) increased chromosome aberrations (3.2), increased SCE (0.65) | | |
| | V79 hamster cells | mutagenic in hprt gene (4.9) | | |
| Amorphous nickel sulfide | Syrian hamster embryo cells | no morphological transformation | IARC 1990 | |
| | CHO cells | no DNA breaks | | |
| Crystalline | Syrian hamster embryo cells | morphological transformation (3.7) | IARC 1990 | |

| Substance | Test system | Result (lowest effective dose in µg Ni/ml) | References |
|-------------------|-----------------------------------|--|-------------------|
| nickel subsulfide | CHO cells | weakly mutagenic in hprt gene (1.1) | Mayer et al. 1998 |
| | rat liver epithelium cells | weakly mutagenic in hprt gene (3.7) | |
| | human lymphocytes | increased frequency of SCE (0.73) | |
| | transgenic rat embryo fibroblasts | no mutagenicity in lacI gene (0.17 mM = 40.8 mg Ni ₃ S ₂ /l) | |

In vivo

The genotoxic properties of nickel compounds *in vivo* are shown in Table 4.

Nickel chloride caused chromosome aberrations in the bone marrow of mice and Chinese hamsters, but it was negative in the dominant lethal test in mice (IARC 1990). Conflicting results were obtained for the induction of micronuclei in the bone marrow of mice. While nickel chloride was positive in one study (Dhir *et al.* 1991), no micronuclei were found for **nickel chloride**, **nickel sulfate** or **nickel oxide** by other groups (Morita *et al.* 1997, Oller and Erexson 2007). **Nickel subsulfide** was not mutagenic in the respiratory tract of transgenic rats or mice (Mayer *et al.* 1998).

Table 4. Genotoxic properties of nickel compounds *in vivo*

| Substance | Species (route of administration) | Dose (mg/kg bw) | Effect | References |
|-------------------|-----------------------------------|---|--|--------------------------------|
| Nickel chloride | Swiss mouse (i.p.) | 6–24 | increased chromosome aberrations in bone marrow cells | Mohanty 1987 |
| | Chinese hamster (i.p.) | 2–10 (4–20% LD ₅₀) | increased chromosome aberrations in bone marrow cells | Chorvatoricova 1983 |
| | mouse (i.p.) | 12.5–100 | negative dominant lethal test, negative micronucleus test | Deknudt and Leonard 1982 |
| | mouse (i.p.) | 10–40 | induction of micronuclei | Dhir <i>et al.</i> 1991 |
| | mouse (i.p.) | 3.2–25 | negative micronucleus test | Dhir <i>et al.</i> 1991 |
| Nickel nitrate | mouse (i.p.) | 56 | negative dominant lethal test, negative micronucleus test | Deknudt and Leonard 1982 |
| Nickel monoxide | mouse (i.p.) | 18.1–145 | negative micronucleus test | Morita <i>et al.</i> 1997 |
| Nickel carbonate | rat (i.p.) | 10–40 | DNA breaks and DNA protein cross links in kidney, but not in liver | Ciccarelli and Wetterhahn 1984 |
| Nickel sulfate | mouse (i.p.) | 5–20 | negative micronucleus test | Morita <i>et al.</i> 1997 |
| | rat (i.p.) | 3–6 (9–14 d) | no increased chromosome aberrations in bone marrow cell or testes | Mathur <i>et al.</i> 1978 |
| | rat (oral) | 125, 250, 500 mg nickel sulfate hexahydrate/kg bw/day | negative micronucleus test | Oller and Erexson 2007 |
| Nickel acetate | mouse (i.p.) | 30 | DNA modifications (³² P-postlabelling) | Chang <i>et al.</i> 1993 |
| | mouse (i.p.) | 16 | oxidative DNA damage in liver and kidneys | Kasprzak <i>et al.</i> 1997 |
| Nickel subsulfide | transgene rat | 4–13 (MTD) | no mutagenicity in the lacI gene | Mayer <i>et al.</i> 1998 |
| | transgenic mouse | nose-only-inhalation | no mutagenicity in the lacI gene | |

Carcinogenicity

Effects in humans

Carcinogenic effects of nickel have long been recognized. The main target is the respiratory system and tumors involve primarily the lungs and nasal cavities. For example, workers employed in a nickel refinery in Clydach, South Wales, during the first two decades of operation (1902 – 1919) had about 6-fold increased risks for lung cancer and about 376-fold increased risks for nasal cancer. The refinery is still in operation, but procedures changed and exposure levels dropped, but an increased cancer risk, albeit to a much lower extent, persisted (summarized in Grimsrud and Peto, 2006). Accordingly, based on several nickel-exposed cohorts from different countries, nickel compounds have been classified by IARC as group 1 carcinogens (IARC, 1990) and as category 1 by EC (see table 1). Thus, while the carcinogenic properties of nickel compounds are widely accepted, several attempts have been undertaken to elucidate the relative contributions of diverse nickel species, i.e. metallic nickel, poorly water soluble nickel sulfide or nickel oxide and water soluble nickel salts.

Metallic (elemental) nickel and nickel alloys

The cohorts with high exposure to nickel metal were generally groups who were simultaneously exposed to other nickel compounds. High exposures to metallic nickel ($\geq 5 \text{ mg/m}^3$) were recorded among workers at calciner furnaces and when cleaning plants in nickel production, where there was a high exposure to oxidic and sulfidic nickel at the same time. An increased incidence of lung and nasal sinus cancers was found in two cohorts of employees who were exposed to nickel metal and other nickel compounds at the same time in the nickel refinery in Clydach, Great Britain, for 15 or more years. By contrast, a cohort of employees of the Oak Ridge Gaseous Diffusion Plant, Tennessee, USA, who had exclusively been exposed to the metallic form of nickel, showed no increased incidence of respiratory tumours. However, the airborne concentration of nickel was relatively low, i.e. below 1 mg/m^3 (Doll 1990, IARC 1990).

Cancer mortality was examined in 31165 employees from 13 plants for the production of high nickel alloys (Arena *et al.* 1998). The authors followed up a cohort of employees from 12 plants (Redmond 1984) through 1988 with inclusion of a further cohort (Enterline and Marsh 1982). No study-specific exposure data were recorded, but only approximate data from experience for the specific work area, which were scattered over a very wide concentration range. The average airborne nickel concentrations were highest in the area of powder metallurgy with 1.5 mg/m^3 , followed by the grinding operation with 0.3 mg/m^3 and the hot working areas with 0.1 mg/m^3 , whereas the means in the other areas were lower. When compared with the cancer mortality data of the total US population, an increased risk of lung cancer mortality was found among workers involved in the production of nickel alloys, but this risk was significantly increased only for employees in the allocated services, i.e. in work areas outside the actual production of alloys in which relatively low nickel concentrations were measured. Analyses of lung cancer mortality in terms of length of employment and time since first employment did not provide a positive association for any work area or for any subcohort defined by sex or race. Since doubts had been

raised about whether the reference population had been selected correctly, the data of the high nickel alloy producers were also compared with two other reference populations, a population in the proximity of the nickel plants (Table 5) and a steel worker cohort from a different study. The authors drew the following conclusions from the study: The patterns of risks for the various work areas and subgroups of sex or race are similar across all three comparison groups. However, the estimated risks are usually lower when local populations are used for comparison. Particularly, no increased risk for lung cancer was noted compared with that of local populations. Although increased risks for colon cancer among non-white males and kidney cancer among white male workers were found compared with all reference groups, there is no strong epidemiological evidence of a causal relation between occupational exposure to high nickel alloys and increased (cancer) mortality.

Table 5. Relative risks (95% confidence intervals) for lung cancer mortality among employees involved in the production and processing of high nickel alloys in the period from 1948–1988 (data from Arena *et al.* 1998)

| Sex/race | Relative risks related to | |
|--------------------------|---------------------------|------------------|
| | US population | Local population |
| Total cohort, n=30,661 | 1.13* (1.06–1.21) | 1.01 (0.95–1.08) |
| White males, n=25,753 | 1.13* (1.05–1.21) | 1.02 (0.96–1.10) |
| Non-white males, n=2,072 | 1.08 (0.85–1.34) | 0.82 (0.66–1.03) |
| Females, n=2,836 | 1.33 (0.98–1.78) | 1.26 (0.94–1.68) |

* p<0.05

Soluble nickel salts

The assessment of soluble nickel as a human carcinogen is based mainly on the increased cancer incidence in two cohorts: a group of workers from Kristiansand, Norway, and a cohort from Harjavalta, Finland (Doll 1990).

There was no significantly increased lung cancer risk in the cohort of 2747 workers in the electrolysis department of the **Port Colborne** refinery (Ontario, Canada). The level of exposure to soluble nickel was estimated to be relatively low there, i.e. 0.25 mg/m³ (compared to ≥ 1 mg/m³ in Kristiansand). The tumour incidence was increased in only one subgroup of employees in Port Colborne, who were also exposed to poorly soluble forms of nickel in the sintering operation (Doll 1990). This result was interpreted by some authors of the cohort study in such a way that exposure to soluble nickel in combination with poorly soluble nickel leads to a promoting effect of soluble nickel (Seilkop 1997).

The **Kristiansand** workers were employed in electrolytic nickel refining using sulphuric acid and were exposed mainly to high concentrations (≥ 1 mg/m³) of nickel sulfate and much lower concentrations of other, poorly soluble nickel compounds. In this group, too, there was a higher incidence of lung and nasal tumours. A recent evaluation of the relation between nickel compounds and respiratory tumours in an extended group of persons who had worked in the nickel refinery in Kristiansand from 1916–1983 substantiated the indications of a carcinogenic

effect of soluble nickel (Andersen *et al.* 1996). A total of 1979 mortalities, 32 new cases of nasal cancer (standardised incidence ratio (SIR) 18.0; 95% confidence interval (CI) 12.3–25.4) and 203 new cases of lung cancer (SIR 3.0; 95% CI 2.6–3.4) were observed. The Kristiansand workers have been further analysed in a case control study with diagnoses occurring 1952-1995 (Grimsrud *et al.*, 2002). In the past, particularly high exposures to soluble nickel occurred in the electrolysis department (Doll 1990). The data seem to be convincing even if exposures to other forms of nickel and sulphuric acid cannot be completely ruled out for this plant either. The data are summarized in Table 6.

Table 6. Relation of lung cancer incidence to the level of exposure to soluble nickel^{a)} (data from Andersen *et al.* 1996)

| Exposure range (mg/m ³) | Mean exposure (mg/m ³) | Cases of lung cancer (n) | Adjusted RR ^{b)} | 95% CI |
|-------------------------------------|------------------------------------|--------------------------|---------------------------|-----------|
| < 1 | 0.1 | 86 | 1.0 | Reference |
| 1–4 | 2.3 | 36 | 1.2 | 0.8–1.9 |
| 5–14 | 8.8 | 23 | 1.6 | 1.0–2.8 |
| ≥ 15 | 28.9 | 55 | 3.1 | 2.1–4.8 |

^{a)} specification of nickel compounds not possible; ^{b)} adjusted for smoking, age, exposure to nickel oxide

A cohort of employees of the Finnish nickel refinery in **Harjavalta** was examined in an extended follow-up of the study of Karjalainen *et al.* (1992) up to December 1995 (Anttila *et al.* 1998). There was an increase in cancer incidence in a cohort of 369 workers with a total of 8794 person years in the electrolytic nickel refinery department between 1960 and 1995. Two cases of nasal cancer were observed in the group of refinery workers exposed primarily towards soluble nickel at mean exposure levels in the order of 0.25 mg Ni/m³ (Anttila *et al.*, 1998). An increased risk of stomach cancer (3 cases; SIR 4.98; 95% CI 1.62–11.6) and lung cancer (6 cases; SIR 2.61; 95% CI 0.96–5.67) was also found. Smelter workers in the same plant with exposure to poorly soluble nickel compounds exerted an increased lung cancer incidence.

No increased lung cancer mortality was found in a cohort of 284 nickel platers in **England**, who had been engaged in the electrolytic nickel plating of car components from 1945–1975 (Pang *et al.* 1996). There were however indications of increased mortality because of stomach cancer. The validity of the study is limited because of the unusually short employment periods (median 0.86 years) and the absence of exposure data.

Sulfidic and oxidic nickel compounds

It is difficult to differentiate between sulfidic and oxidic compounds in epidemiology since generally sulfides are calcined to oxides in nickel-producing plants. Altogether, there were increased risks of lung and nasal cancers among persons exposed to sulfidic and oxidic nickel. One example is the Clydach cohort. It consisted of 2521 men which were employed in various processes in the refining of nickel from grinding and roasting of nickel containing sulfidic nickel and copper, extraction of copper by sulfuric acid, reduction of oxidic nickel by water gas,

extraction of nickel as gaseous nickel carbonyl and decomposition of the carbonyl by heat. Workers were predominantly exposed to sulfidic, oxidic and metallic nickel dusts and, to a lower extent, to soluble nickel salts in a hydrometallurgical plant. Reliable quantitative data about airborne exposures do not exist, except the fact that exposures had been very high in milling/grinding, roasting and plant cleaning occupations. There were high incidences of lung and nasal cancers in workers hired prior to 1930 (Doll 1990). This sub-cohort exhibited 172 lung cancer cases with a SMR 393 (95% CI 336-456) and 74 nasal cancers with a SMR 21119 (95% CI 16583-26514). Due to improved factory hygiene and personal protective gear, workers hired after 1930 had significantly lower cancer risks, and those hired after 1940 showed little if any increased lung or nasal cancer risk (Doll 1990).

When the tumour incidences are compared between groups exposed to other nickel compounds to a high or low extent, there is an indication of sulfidic nickel being a carcinogen: Among workers with relatively low exposure to oxidic and soluble nickel, but high exposure to sulfidic nickel, the lung cancer risk was clearly higher than among those with lower exposure to sulfidic nickel (Doll 1990). The finding that workers exposed mainly to oxidic nickel showed an increased incidence of lung and nasal cancers is an indication of the carcinogenicity of oxidic nickel (Doll 1990). The Clydach cohort was followed until 2000 (Grimsrud and Peto, 2006).

Altogether, epidemiological evidence points towards a dose-related carcinogenic potential of water soluble nickel compounds, especially evident also after quantitative reevaluation of the Kristiansand cohort (Norway) (Grimsrud et al., 2002), the Clydach cohort (South Wales) (Easton et al., 1992) and the Harjavalta cohort (Finland) (Antilla et al., 1998). Concerning the water insoluble nickel species, reevaluation of the Clydach cohort revealed that at least one insoluble nickel species (oxidic, sulfidic or metallic) contributed to cancer risk (Easton et al., 1992). A general contribution, albeit not dose-dependent, of sulfidic and oxidic nickel species to cancer risk was also seen in the reevaluation of the Kristiansand cohort, while no impact on carcinogenicity was found for metallic nickel (Grimsrud et al., 2002).

The epidemiological evidence with respect to the carcinogenic potential of water soluble nickel compounds appears to contradict the results of the animal study in which the inhalation of nickel sulfate induced no tumours in rats or mice (Dunnick *et al.* 1995, NTP 1996c). This discrepancy might be explained by the high toxicity of water soluble nickel sulfate in rats and mice. Thus, in the NTP study only a concentration of up to 0.1 mg/m³ was used in rats, and up to 0.2 mg/m³ in mice could be applied, whereas the respiratory tract tumours among workers exposed to soluble nickel compounds were recorded only at concentrations of 0.25 mg/m³ and higher.

It has to be emphasized, however, that the epidemiological evaluation of the carcinogenic risk for different nickel species has some limitations. Thus, there are no cohorts available exclusively exposed to a single nickel species. Furthermore, assessments of the relative contribution of the diverse nickel species far back in time depend largely on exposure estimates such as job history, which introduces uncertainty, and comparatively minor differences may have a high impact on

dose-response relationships. This is especially true since high exposures with clearly elevated cancer risks were found for workers first employed before 1930 for example in the Clydach refinery (Grimsrud and Peto, 2006). Finally, combination effects either with confounding factors (smoking, sulphuric acid in case of Kristiansand) or between water soluble and water insoluble nickel species cannot be excluded.

Effects in experimental animals

Nickel metal

Inhalation: A recently conducted inhalation study with male and female Wistar rats yielded no significant increase in lung tumors at exposure levels of 0.1 and 0.4 mg/m³ nickel powder. There was, however, a significant exposure-related increase in pheochromocytomas of the adrenal medulla in male rats as well as a significant increase in adenomas and carcinoma of the adrenal cortex in female rats (Oller et al. 2008; see Table 7). Nevertheless, the significance of these endpoints for human carcinogenicity is presently unknown and underlying mechanisms imply that comparatively high concentrations are required to exert these effects.

Table 7 Incidences (percentages) of adrenal gland tumors in rats exposed to nickel metal by inhalation (Oller et al. 2008)

| | Concentration (mg Ni/m ³) | | | | | |
|---|---------------------------------------|-----------|------------|------------|---------------------|--------------------|
| | 0 | | 0.1 | | 0.4 | |
| | males | Females | males | females | males | females |
| Pheochromocytoma (adrenal medulla) | | | | | | |
| Benign | 0/50 | 0/50 | 5/50 (10%) | 5/49 (10%) | 19/50 (38%)* | 3/53 (6%) |
| Malignant | 0/50 | 0/50 | 0/50 | 0/49 | 5/50 (10%)* | 0/53 |
| Combined | 0/50 | 0/50 | 5/50 (10%) | 5/49 (10%) | 21/50 (42%)* | 3/53 (6%) |
| Adrenal Cortex | | | | | | |
| Adenoma | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 2/49 (4%) | 2/50 (4%) | 4/54 (7%) |
| Carcinoma | 0/50 | 1/50 (2%) | 0/50 | 0/49 | 0/50 | 3/53 (6%) |
| Combined | 1/50 (2%) | 2/50 (4%) | 3/50 (6%) | 2/49 (4%) | 2/50 (4%) | 7/54 (13%)* |

* statistically significant according to Peto method

Intratracheal instillation: Intratracheal instillation of nickel powder (99.9% nickel) induced malignant lung tumours in female Wistar rats, which did not occur in control animals. The number of animals with tumours was 10 of 39 at a dose of 6 mg distributed over 20 administrations and 8 of 32 at 9 mg distributed over 10 administrations; the control was 0 of 40 animals (Pott *et al.* 1987).

In hamsters, the intratracheal instillation of nickel powder (99.9%) did not lead to a significant increase in the incidence of lung carcinomas (Muhle *et al.* 1990).

Intraperitoneal injection: Intraperitoneal injection of nickel powder (100% nickel) induced local, malignant lung tumours in female Wistar rats in a dose-dependent manner. A single dose of 6 mg nickel induced tumours in 4 of 34 animals, 12 mg nickel in 2 doses induced tumours in 5 of 34 and 25 mg nickel over 25 administrations induced tumours in 25 of 35 animals (Pott *et al.* 1992). 75 mg distributed over 10 administrations caused malignant tumours in 46 of 48 animals, while in the control 5 of 204 animals showed tumours (Pott *et al.* 1987). High nickel alloys induced malignant tumours in relation to the dose in the same test series. An alloy with 74% nickel (16% chromium, 7% iron and < 0.2% cobalt) and an alloy with 50% nickel (remainder aluminium) were carcinogenic, whereas an alloy with 32% nickel (21% chromium, 55% iron, 0.8% manganese and < 0.04% cobalt) resulted in no significantly increased tumour incidence (Pott *et al.* 1990).

Subcutaneous or intramuscular injections, which also caused local tumours, are not considered here since the relevant results are generally not adequately substance-specific.

Conclusion: Elemental nickel caused malignant lung tumours after intratracheal instillation and intraperitoneal injection in rats, but not after chronic inhalation. Nevertheless, there was a pronounced increase in benign and malignant pheochromocytomas especially in male rats as well as a significant increase in adenomas and carcinomas of the adrenal cortex in females at the highest dose group (0.4 mg Ni/m³).

Soluble nickel(II) salts (nickel acetate, nickel sulfate): Local tumours were induced by soluble nickel acetate injected intraperitoneally (Pott *et al.* 1992). The combination of nickel acetate (intraperitoneal) with the promoter sodium barbital in the drinking water caused renal tumours in rats (Kasprzak *et al.* 1990). In the only published inhalation study with nickel sulfate that was carried out within the NTP, no respiratory tract tumours were induced in rats or mice (Dunnick *et al.* 1995, NTP 1996c). The concentrations of nickel sulfate were, however, limited to 0.11 mg/m³ in rats and 0.22 mg/m³ in mice, since toxicity (pneumonia) occurred at higher doses (in comparison: According to the results of studies of exposed persons, the carcinogenic airborne concentrations of soluble nickel were in most cases above 1 mg/m³).

Conclusion: The only animal inhalation study with a soluble nickel salt yielded negative results. The apparent contradiction with the epidemiological findings can possibly be explained by considering that the respiratory tract tumours among workers were recorded only after high exposures to about 0.25 mg nickel/m³ as soluble nickel, whereas the exposure in animal experiments was limited to a maximum concentration of 0.11 mg nickel/m³ (rats) or 0.22 mg nickel/m³ (mice) as nickel sulphate because of severe local toxicity.

Oxidic nickel: Nickel monoxide induced lung tumours in rats after intratracheal instillation (Pott *et al.* 1987). After inhalation of nickel monoxide in rats in the NTP study, a higher incidence of lung tumours was found in male and female rats in relation to the dose, whereas

there was no clear relation to the dose in female mice nor were there any neoplastic effects in male mice (Dunnick *et al.* 1995, NTP 1996a).

Sulfidic nickel: Crystalline, but not amorphous nickel monosulfide induced local tumours in the injection area in rats (IARC 1990). In an early inhalation study (Ottolenghi *et al.* 1974) and in a more recent inhalation study under the NTP (Dunnick *et al.* 1995, NTP 1996b), nickel subsulfide was shown to be a clear lung carcinogen in rats, but not in mice.

Investigations on the carcinogenicity of nickel and nickel compounds are summarized by IARC (1990). The studies relevant for evaluation are shown in Table 8.

Conclusion: Nickel monoxide and nickel subsulfide were found to be carcinogenic by inhalation in animal studies.

Table 8. Studies on the carcinogenicity of nickel and nickel compounds in experimental animals

| Species, sex | Route of administration, study duration | Dose | Tumour incidence | Type of tumour ¹⁾ | References |
|------------------------------|---|--|------------------|---|--------------------------|
| Nickel metal | | | | | |
| Wistar rat, male | inhalative, 6h/d, 5d/w, 2 years | Controls | 0/50 | benign and malignant pheochromocytoma | Oller <i>et al.</i> 2008 |
| | | 0.1 mg/m ³ | 5/50 | | |
| | | 0.4 mg/m ³ | 21/50* | | |
| Wistar rat, female | inhalative, 6h/d, 5d/w, 2 years | Controls | 2/50 | adenoma and carcinoma of the adrenal cortex | Oller <i>et al.</i> 2008 |
| | | 0.1 mg/m ³ | 2/49 | | |
| | | 0.4 mg/m ³ | 7/54* | | |
| Wistar rat, female | intratracheal, 2.5 years | Controls | 0/40 | malignant lung tumours | Pott <i>et al.</i> 1987 |
| | | 20 x 0.36 mg Ni/rat, once weekly | 10/39** | | |
| | | 10 x 0.9 mg Ni/rat, once weekly | 8/32** | | |
| Wistar rat, female | i.p., 2 years | Controls | 4/133 | local malignant tumours | Pott <i>et al.</i> 1992 |
| | | 1 x 6 mg Ni/rat | 4/34* | | |
| | | 2 x 6 mg Ni/rat | 5/34* | | |
| | | 25 x 1 mg Ni/rat | 25/35** | | |
| Syrian hamster, male, female | intratracheal, 26 - 30 month | Controls | 0/60 | adenocarcinoma | Muhle <i>et al.</i> 1990 |
| | | 12 x 0.8 mg Ni/hamster at 14 day intervals | 1/60 | | |
| Nickel acetate | | | | | |
| Wistar rat, female | i.p., 2 years | Controls | 1/33 | malignant lung tumours | Pott <i>et al.</i> 1992 |
| | | 25 x 1 mg Ni/rat, once weekly | 3/35 | | |
| | | 50 x 1 mg Ni/rat, twice weekly | 5/31* | | |

| | Route of administration, study duration | Dose | Tumour incidence | Type of tumour ¹⁾ | References |
|-------------------------------------|---|--|---|--|----------------|
| Nickel sulfate (hexahydrate) | | | | | |
| Fischer rat, female | inhalative, 6h/d, 5d/w, 2 years | Controls 0.11 mg Ni/m ³ (MTD) | 0/52 1/54 | adenoma (NTP: "no evidence") | NTP 1996 c |
| Fischer rat, male | inhalative, 6h/d, 5d/w, 2 years | Controls 0.11 mg Ni/m ³ (MTD) | 2/54 3/53 | adenoma + carcinoma (NTP: "no evidence") | NTP 1996 c |
| B6C3F1 mouse, female | inhalative, 6h/d, 5d/w, 2 years | Controls 0.22 mg Ni/m ³ (MTD) | 7/61 2/60 | adenoma + carcinoma (NTP: "no evidence") | NTP 1996 c |
| B6C3F1 mouse, male | inhalative, 6h/d, 5d/w, 2 years | Controls 0.22 mg Ni/m ³ (MTD) | 13/61 8/61 | adenoma + carcinoma (NTP: "no evidence") | NTP 1996 c |
| Nickel(II) oxide | | | | | |
| Fischer rat, female | inhalative, 6h/d, 5d/w, 2 years | Controls 0.5 mg Ni/m ³ 1.0 mg Ni/m ³ 2.0 mg Ni/m ³ | 1/53 0/53 6/53 (p=0.056) | adenoma + carcinoma (NTP: " some evidence ") | NTP 1996 a |
| Fischer rat, male | inhalative, 6h/d, 5d/w, 2 years | Controls 0.5 mg Ni/m ³ 1.0 mg Ni/m ³ 2.0 mg Ni/m ³ | 1/54 1/53 6/53 (p=0.053) 4/54 | adenoma + carcinoma + squamous cell carcinoma (NTP: " some evidence ") | NTP 1996 a |
| B6C3F1 mouse, female | inhalative, 6h/d, 5d/w, 2 years | Controls 1.0 mg Ni/m ³ 2.0 mg Ni/m ³ 3.9 mg Ni/m ³ | 6/64 15/66* 12/63 (p=0.095) 8/64 | adenoma + carcinoma (NTP: " equivocal evidence ") | NTP 1996 a |
| B6C3F1 mouse, male | inhalative, 6h/d, 5d/w, 2 years | Controls 1 mg Ni/m ³ 2 mg Ni/m ³ 3.9 mg Ni/m ³ | 9/57 14/67 15/66 14/69 | adenoma + carcinoma (NTP: "no evidence") | NTP 1996 a |
| Nickel sulfide | | | | | |
| Fischer rat, male | i.m., 2 years crystalline amorphous | Control 1 x 14 mg Ni/rat 1 x 14 mg Ni/rat | 0/84 14/14** 3/25* | local sarcomas | Sunderman 1984 |

| Species, sex | Route of administration, study duration | Dose | Tumour incidence | Type of tumour ¹⁾ | References |
|--------------------------|---|--|--|--|-------------------------------|
| Nickel subsulfide | | | | | |
| Fischer rat, female | inhalative, 6 h/d, 5d/w, 80 weeks | Controls 0.73 mg Ni/m ³ | 1/107 12/98** | adenoma + carcinoma | Ottolenghi <i>et al.</i> 1974 |
| Fischer rat, male | inhalative, 6 h/d, 5d/w, 80 weeks | Controls 0.73 mg Ni/m ³ | 1/108 17/110* | adenoma + carcinoma + sarcoma | Ottolenghi <i>et al.</i> 1974 |
| Fischer rat, female | inhalative, 6 h/d, 5d/w, 2 years | Controls 0.11 mg Ni/m ³ 0.73 mg Ni/m ³ | 2/53 3/53 9/53* | adenoma + carcinoma (NTP: " clear evidence ") | NTP 1996 b |
| Fischer rat, male | inhalative, 6 h/d, 5d/w, 2 years | Controls 0.11 mg Ni/m ³ 0.73 mg Ni/m ³ | 0/53 6/53* 11/53** | adenoma + carcinoma (NTP: " clear evidence ") | NTP 1996 b |
| B6C3F1 mouse, female | inhalative, 6 h/d, 5d/w, 2 years | Controls 0.44 mg Ni/m ³ 0.88 mg Ni/m ³ | 9/58 2/59 3/60 | adenoma + carcinoma (NTP: "no evidence") | NTP 1996 b |
| B6C3F1 mouse, male | inhalative, 6 h/d, 5d/w, 2 years | Controls 0.44 mg Ni/m ³ 0.88 mg Ni/m ³ | 13/61 5/59 6/58 | adenoma + carcinoma (NTP: "no evidence") | NTP 1996 b |

¹⁾ lung tumours, unless otherwise stated; * p<0.05 Fischer exact test; ** p<0.01 Fischer exact test

Mode of Action

An integrating consideration of the relevant cellular and biochemical findings allows the conclusion that the release of nickel ions is responsible for the genotoxic and carcinogenic effects of all forms of nickel. Additionally, the long half life of insoluble particles contributes to the inflammatory effects in the lung. These inflammatory effects are not unspecific particle effects, since they occur at much lower concentrations as compared to other biopersistent particles such as titanium dioxide.

Nickel ions from readily soluble nickel salts are slowly taken up via ion channels in cell membranes. The less soluble metallic, sulfidic and oxidic forms of nickel are taken up in mammalian cells by phagocytosis, and due to the low pH, are gradually dissolved in lysosomes, yielding high concentrations of nickel ions in the nucleus. Thus, metallic (elemental) nickel was phagocytosed by alveolar macrophages of exposed rats (Johansson *et al.* 1980) and also *in vitro* by CHO cells (Costa and Mollenhauer 1980). Nickel subsulfide was phagocytosed by CHO cells (Lee *et al.* 1995). Intracellular distribution in cultured CHO and A549 human lung cells was examined in detail for some poorly soluble nickel compounds as compared to water soluble nickel compounds (Costa *et al.*, 1981; Harnett *et al.*, 1982; Schwerdtle and Hartwig, 2006). Thus, in A549 cells, both water soluble nickel chloride and particulate black nickel oxide lead to

a time- and dose-dependent increase in nickel bound to cytoplasmic and nuclear proteins; the concentrations reached in the nucleus after incubation with nickel oxide were about twofold higher as compared to nickel chloride (Schwerdtle and Hartwig, 2006). After phagocytosis of nickel subsulfide, stable ternary protein-nickel-DNA complexes were formed in the nuclei of CHO cells (Lee *et al.* 1982).

Higher nuclear concentrations after phagocytosis combined with a longer tissue half-life in case of poorly soluble nickel compounds compared with that of readily soluble ones is one important aspect to understanding the more severe chronic toxic effects including carcinogenicity of the poorly soluble compounds compared with the readily soluble ones in experimental animals. However, in humans soluble nickel compounds appear to be stronger carcinogens as compared to less soluble and metallic species. This discrepancy is likely due to the high toxicity of readily soluble nickel compounds in animals, which did not allow the application of concentrations relevant for human exposure.

According to mechanistic studies, nickel ions are the ultimate genotoxic forms of nickel. Soluble nickel salts are non-mutagenic in almost all bacterial mutagenicity tests and only weakly mutagenic in tests with mammalian cells. Nickel ions cause chromosome aberrations, sister chromatid exchange, DNA breaks and DNA–protein cross links in mammalian cells only in higher concentrations (mmol/l range) (IARC 1990).

The genotoxicity and carcinogenicity appears not to be mediated by direct interaction with DNA but rather indirectly by an increased formation of reactive oxygen species, by interaction with proteins involved in maintaining genomic stability and by epigenetic mechanisms altering gene expression profiles.

(1) Enhanced formation of reactive oxygen species catalyzed by nickel: In the presence of hydrogen peroxide, nickel(II) ions produce oxidative DNA damage to isolated DNA and isolated chromatin, these effects being reduced by antioxidants (Kasprzak and Hernandez 1989, Lloyd and Philips 1999). With certain peptides (e.g. Gly-Gly-His), nickel complexes also function *in vitro* as catalysts for the formation of hydroxyl radicals from hydrogen peroxide, similarly to the Fenton reaction catalyzed by iron (Torreilles *et al.* 1990). Nickel complexes with histones enhance the formation of oxidized guanine in DNA by hydrogen peroxide (Nackerdien *et al.* 1991) or atmospheric oxygen (Bal *et al.* 1996). However, overall oxidative DNA damage was observed in cell cultures only in cytotoxic nickel chloride concentrations (≥ 0.75 mM) (Dally and Hartwig 1997). At similar concentrations, DNA-protein crosslinks were induced by nickel subsulfide and nickel sulphate in isolated rat lymphocytes; these were due to the formation of reactive oxygen species (Chakrabarti *et al.*, 2001; Wozniak and Blasiak, 2002). An increase in 8-oxo-dG was also observed in rat lungs after intratracheal instillation of 1 mg Ni₃S₂, NiO or NiSO₄. However, analysis was only performed 48 h after treatment; thus, the increase may be due to either induction of oxidative DNA damage or to DNA repair inhibition of endogenous oxidative DNA damage. Furthermore, inflammation was observed under these conditions as well, giving rise to secondary genotoxicity (Kawanishi *et al.*, 2002).

(2) Epigenetic mechanisms inducing increased cell proliferation: Nickel chloride altered the expression of various genes in CHO cells (Costa and Klein 1999, Lee *et al.* 1999, Mollerup *et al.* 1996, Salnikow *et al.* 2000, Zhou *et al.* 1998). For example, nickel compounds have been shown to up-regulate a battery of hypoxia-inducible genes. As underlying mechanism, nickel-induced degradation of ascorbate, subsequent iron oxidation and thus inhibition of prolyl hydroxylases have been postulated. This leads finally to the inactivation of the von Hippel-Lindau tumor suppressor protein (Salnikow *et al.*, 2004). Furthermore, effects on chromatin structure and function have been described. Thus, nickel chloride caused increased methylation of cytosine bases in tumour suppressor genes resulting in their inactivation with increased cell proliferation (Lee *et al.* 1995, 1998). Furthermore, it inhibited histone acetylation and caused chromatin condensation with reduced expression of marker genes (Salnikow *et al.* 1994; Broday *et al.* 1999; 2000,).

(3) Inhibition of the repair of DNA damage which is generated by direct mutagens, but is also always present as background, for example oxidative DNA damage. This mechanism leads to an increase of genotoxicity in combination with other DNA-damaging agents. Nickel ions enhance the transformation of hamster embryo cells by benzo[a]pyrene, the mutagenicity of methyl methanesulfonate in *E. coli* as well as the mutagenicity and induction of sister chromatid exchanges by UV radiation in hamster cells (IARC 1990). The inhibition of DNA repair processes was identified as the mechanism of action on which this enhancement is based. The main DNA repair systems are affected by this inhibition: Nucleotide excision repair involved in the removal of bulky DNA damage induced predominantly by environmental mutagens, base excision repair involved in the removal of DNA base damage induced for example by reactive oxygen species and the O⁶-methylguanine-methyltransferase (MGMT) repairing almost exclusively O⁶-methylguanine induced by alkylating agents. (Dally and Hartwig 1997, Hartwig *et al.* 1994, Iwitzki *et al.* 1998, Krueger *et al.* 1999, Schwerdtle *et al.*, 2002). The degradation of the promutagenic DNA precursor 8-oxo-dGTP by a specific GTPase is also inhibited by nickel(II) (Porter *et al.* 1997). As potentially very sensitive targets so called zinc finger proteins have been identified, where zinc is replaced by nickel which increases the vulnerability of complexing thiol groups towards oxidation (Kopera *et al.*, 2004). These structures are found in several proteins involved in DNA repair and cell cycle control; their inactivation may lead to severe disturbances in the cellular response to DNA damage. A co-mutagenic effect of nickel ions also agrees with the results of epidemiology, since lung tumor incidence was greatly increased in combination with tobacco smoke (Andersen *et al.* 1996).

(4) A non-substance-specific mechanism of carcinogenicity based on a particle overload effect was suggested for nickel oxide (Oller *et al.* 1997). According to the authors, the poorly soluble nickel oxide leads to chronic activation of macrophages resulting in chronic inflammation and being carcinogenic only secondarily. In general, the concentrations of other substances at which carcinogenic effects by particle overload occur are clearly higher than those of nickel oxide. While nickel oxide induced tumours in rats at concentrations as low as 1.25 mg/m³ (Dunnick *et al.* 1995), lung tumours were induced at 18 mg/m³ for talcum (NTP 1993) and at 45 mg/m³ for antimony trioxide (Groth *et al.* 1986), and the lowest carbon black concentration used in a chronic inhalation study where lung tumours were induced was 2.5 mg/m³, with rats being

exposed for 16 hours/day, 5 days/week for 2 years (Mauderly *et al.*, 1994). The general consensus is that lung tumours seen in rats under conditions of particle overload can occur with a range of respirable poorly-soluble low-toxicity substances and that their lung tumour-inducing potency is more closely related to particle size and particle-surface properties rather than mass (Greim *et al.*, 2001). In the case of nickel oxide, it cannot be regarded as a toxicologically-inert particle and thus, although the particle overload effect may induce some chronic activation of macrophages with the concomitant production of reactive oxygen species and thus contribute to the carcinogenic process, it does not seem likely that this will account for all the total carcinogenic process causing lung cancers seen in the Dunnick *et al.* 1995 study.

Reproductive Toxicity

Effects on fertility

An increase in abnormalities was observed in spermatozoa from mice treated orally with a single dose of **nickel chloride** (43 mg Ni/kg bw). Dose related effects on sperm motility and count as well as decreased body weight gain were observed after repeated dosing with nickel chloride at 10 and 20 mg/kg bw/day, but not at a dose level of 5 mg/kg bw/day. However, due to the limited number of animals used in this study, the dose level of 5 mg/kg bw/day cannot be considered as a reliable NOAEL (EU-RAR 2008d).

No effects on sperm morphology or motility, or on vaginal cytology, were observed in rats or mice exposed to concentrations up to 0.45 mg Ni/m³ as **nickel sulfate hexahydrate** for 6 h/day, 5 days/week for 13 weeks (Dunnick *et al.*, 1989; NTP, 1996a). In addition, no histopathological effects on reproductive tissue were observed in the chronic studies, with exposures at concentrations up to 0.11 mg Ni/m³ (rats) or 0.22 mg Ni/m³ (mice) for 6 h/day, 5 days/week for 2 years. Degeneration of the germinal epithelium of the testes was observed only at the much higher concentration of 1.6 mg Ni/m³ in male rats exposed for 6 h/day for 12 days over a 16-day period (Benson *et al.*, 1988) (Haber *et al.* 2000).

Smith *et al.* (1993; see: EU-RAR 2008d) conducted a 1-generation reproductive toxicity study with female Long Evans rats (34/dose) administered 0, 10, 50, or 250 ppm nickel as **nickel chloride hexahydrate** in drinking water starting 11 weeks prior to breeding; mating was performed with unexposed males. The overall average doses were reported as 0, 1.33, 6.80, and 31.63 mg Ni/kg bw/day. A small, but statistically significant decrease in prolactin was observed in high-dose dams. There was no treatment-related effect on reproductive performance indices (mating success, rate of impregnation) but pup mortality was observed at all doses (see below). The NOAEL for fertility in this study is the highest dose of 31.63 mg Ni/kg bw/day and a LOAEL was not identified. However, it should be noticed that effects on sperm quality and oestrus cyclicity were not investigated in this study.

RTI (1988; see: EU-RAR 2008d) administered **nickel chloride hexahydrate** to male and female CD rats (30/sex/dose) at concentrations of 0, 50, 250, or 500 ppm nickel in drinking water in a 2-generation study. The parental animals were exposed beginning 11 weeks before cohabitation. The estimated doses were 0, 6.0, 25, and 42 mg Ni/kg bw/day. There was no treatment-related effect on reproductive performance indices (mating success, rate of

impregnation), reproductive organ weights or histopathology of reproductive organs but pup mortality was observed at all dose levels (see below). The NOAEL for fertility in this study is the highest dose of 42 mg Ni/kg bw/day and a LOAEL was not identified. However, it should be noticed that effects on sperm quality and oestrus cyclicity were not investigated in this study.

A range-finding one-generation study in Sprague-Dawley rats was performed prior to the two-generation study described below (EU-RAR 2008b). Groups of 8 males and 8 females were given **nickel sulphate hexahydrate** at doses of 0, 10, 20, 30, 50, 75 mg/kg bw/day by gavage. Dosing began two weeks prior to mating and dosing of F1 began on postnatal day 21. These doses had no effects on F0 survival, growth, gross necropsy findings or fertility but mortality of the pups occurred at all dose levels (see below). However, as a limited number of animals per group was used a clear NOAEL for fertility of 75 mg/kg bw/day (16.8 mg Ni/kg bw/day) cannot be established based on these results.

In a 2-generation reproduction study compliant with the OECD 416 test guidelines, Sprague-Dawley rats were administered **nickel sulphate hexahydrate** at dose levels of 1, 2.5, 5.0, and 10 mg/kg bw/day by gavage (EU-RAR 2008b). There were no effects on fertility, sperm quality, oestrous cyclicity or sexual maturation and no other treatment-related signs. Pup mortality was slightly but statistically not significantly increased at 10 mg/kg bw/day. Since the highest dose level did not induce any signs of toxicity in the F0 animals, the study does not fulfil OECD TG guidelines concerning the dose levels used. Therefore, the results of the study are not conclusive concerning the potential for effects of nickel sulphate on fertility at higher dose levels than 10 mg/kg bw/day (2.2 mg Ni/kg bw/day).

In a 3-generation reproduction study in Wistar rats (EU-RAR 2008b) groups of 30 weanling rats per sex per group were fed 0, 250, 500, or 1000 ppm nickel (roughly 0, 13-20, 26-40 and 52-80 mg Ni/kg bw/day) as **nickel sulphate hexahydrate** for 11 weeks. A complete histopathology examination was conducted on F3b weanlings (10/sex/group). Even though the body weights of the F0 rats were slightly decreased at the high dose and the fertility index was slightly lower at 250 and 1000 ppm in the F1a generation, and at 1000 ppm in the F2b generation, the differences were not statistically significant. The fertility index in exposed animals was similar to control values at the high dose in F1b, F2a, F3a and F3b. The number of pups born dead was increased at all nickel doses (see below). Based on the results of the study, the NOAEL for effects on fertility appears to be 1000 ppm (52-80 mg Ni/kg bw/day), but due to the limited reporting of the data there is uncertainties concerning this NOAEL.

Developmental toxicity

Prenatal evaluation

No valid studies on prenatal developmental toxicity of nickel and nickel compounds are available.

A teratological evaluation was performed in F2b foetuses in a two-generation study with administration of **nickel chloride hexahydrate** to male and female CD rats at 0, 50, 250, or 500 ppm nickel in drinking water (EU-RAR 2008d; see below). The percent foetuses malformed per litter were significantly increased at 50 ppm, due primarily to a higher incidence of short rib in

that group. In the absence of similar effects at higher doses, the increased incidence at 50 ppm is probably not due to exposure to nickel. Increased neonatal mortality was observed at 500 ppm (42 mg Ni/kg bw/day).

Studies using intraperitoneal or intramuscular dosing (presumably of nickel chloride) during pregnancy in mice and rats have reported reduced number of live pups, lower body weights in foetuses and offspring, or malformations. These studies are not considered useful for risk assessment because of the route of exposure (EU-RAR 2008d).

According to an abstract from Morvai *et al.* (1982), the group has previously reported that **nickel sulphate** is embryotoxic and teratogenic in mice and rats, and it is embryotoxic and induces spontaneous abortion in rabbits. These studies have, however, not been located in published literature. The abstract describes a study where groups of nonpregnant and pregnant rats were treated daily for 10 days or between the 6th and 15th days of the organogenesis with 100 mg nickel sulphate /kg bw/day (22 mg Ni/kg bw/day) by gavage. Authors concluded that nickel caused embryotoxic and teratogenic effects. The results reported from this study indicate that a dose level of 100 mg/kg bw/day (22 mg Ni/kg bw/day) may cause malformations. However, the study is only reported in an abstract and the findings can therefore not be properly evaluated (EU-RAR 2008b).

In a study that evaluated effects on developmental or reproductive function after inhalation of NiO, Weischer *et al.* (1980) exposed groups of 10–13 pregnant Wistar rats continuously to NiO at 0.8, 1.6, or 3.2 mg compound/m³ (0.6, 1.2, or 2.5 mg Ni/m³) for 21 days, beginning on gestation day 1. Maternal endpoints evaluated were body weight, organ weights, serum urea, and hematology. The only fetal endpoints evaluated were fetal weight, leukocytes, and serum urea. Maternal body weight gain was statistically significantly reduced in all exposed groups, and statistically significant decreases in fetal body weight were observed at the top two exposure levels. Fetal weight was significantly decreased at the mid- and high-concentration level. Other developmental effects, such as fetal survival, were apparently not evaluated (Haber *et al.* 2000).

Postnatal Evaluation

Smith *et al.* (1993; see: EU-RAR 2008d) conducted a 1-generation reproductive toxicity study (with two breedings) of female Long Evans rats administered 0, 10, 50, or 250 ppm nickel as **nickel chloride hexahydrate** in drinking water (see above). The overall average doses were reported as 0, 1.33, 6.80, and 31.63 mg Ni/kg bw/day. Statistically significant decreases in maternal body weight gain during gestation were observed at the mid and high dose groups. There was no treatment-related effect on mean pup birth weight or weight gain in either generation. However, there was a dose-related increase in both the number and proportion per litter of pups either born dead or dying shortly thereafter. The total number of dead pups and the proportion of dead pups per litter were significantly increased at the high dose in both the first and second breeding. There was no effect on other measures of pup mortality in the first generation, but the total number of dead pups and the percentage of dead pups per litter on postnatal day 1 were statistically significantly increased at all doses in the second generation, and the number of litters with dead pups was also borderline significant at the low dose of the second generation. The inconsistency between generations makes it difficult to identify a clear

NOAEL or LOAEL for this study. However, as all three measures of pup death were statistically significant or borderline significant at the low dose in the second generation an **equivocal LOAEL for this study was 1.33 mg Ni/kg bw/day**.

RTI (1988; see: EU-RAR 2008d) administered **nickel chloride hexahydrate** to male and female CD rats at 0, 50, 250, or 500 ppm nickel in drinking water in a 2-generation study. The estimated doses were 0, 6.0, 25, and 42 mg Ni/kg bw/day for the Po generation and 0, 6.2, 23, and 42 mg Ni/kg bw/day for the F1 adult generation. At the 500 ppm dose level there was a statistically significant decrease in the adult Po and F1 body weight, along with decreased absolute and relative liver weights in Po females. Thus, 250 ppm (25 mg Ni/kg bw/day) was a NOAEL for adult animals. In the F1a, b and F2a generation at the 500 ppm dose level, the number of live pups/litter was significantly decreased, pup mortality was significantly increased, and average pup body weight was significantly decreased in comparison with controls. No effects on prenatal growth or viability were observed in F2b. Although there was no statistically significant effect at 250 ppm in F1a, there was some indication of decreased number of live pups/litter. In the 50 and 250 ppm dose groups, increased pup mortality and decreased live litter size was observed in the F1b litters. However, these effects seen in the F1b litters are somewhat questionable because the room temperature was 3-5° C higher than normal at certain times (gestation, postnatal days) along with lower levels of humidity. Therefore, the above results seen at 50 and 250 ppm may not be adverse effects of nickel only. Overall, the study shows that exposure to nickel can cause increased neonatal mortality at 42 mg Ni/kg bw/day and possibly at lower doses of 6 and 25 mg Ni/kg bw/day, but a reliable developmental NOAEL cannot be identified in this study.

In the range-finding one-generation study in Sprague-Dawley rats given **nickel sulphate hexahydrate** at doses of 0, 10, 20, 30, 50, 75 mg/kg bw/day by gavage. (EU-RAR 2008b; see above), evaluation of postimplantation/perinatal lethality among the offspring of treated parental rats (i.e. number of pups conceived minus the number of live pups at birth) showed statistically significant increases at the 30, 50, and 75 mg/kg bw/day exposures. The values were also increased at the 10 and 20 mg/kg bw/day levels, however, the difference was not statistically significant. The mean live litter size was significantly decreased at 75 mg/kg bw/day. The number of dead offspring on lactation day 0 (stillbirth) was significantly increased in all exposure groups except the 50 mg/kg bw/day group. The results of this range-finding study indicate a LOAEL for neonatal death of 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) and a NOAEL was not found.

In the 2-generation reproduction study in Sprague-Dawley rats administered **nickel sulphate hexahydrate** at dose levels of 1, 2.5, 5.0, and 10 mg/kg bw/day by gavage (EU-RAR 2008b; see above) the postimplantation/perinatal lethality until postnatal day 0 among the F1 offspring (i.e. number of pups conceived minus the number of live pups at birth) was higher at 10 mg/kg bw/day, however, the difference was not statistically significant (2.1 at 10 mg/kg bw/day vs. 0.9 in the control group, $p = 8.6\%$ in Mann-Whitney test). In F2 offspring, the value for postimplantation/perinatal lethality was similar to the F2 control value. The authors state that the results indicate that the highest dose of 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) was a NOAEL for the developmental end points studied, including the variable of postimplantation/perinatal lethality. Based on supplementary statistics using the litter as the statistical unit and showing

that the increase in postimplantation/perinatal lethality in F1 is statistically significant as well as the above consideration concerning the finding of effects in F1 but not in F2, 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) cannot be regarded as a clear NOAEL. Consequently, the **NOAEL is set to 5 mg/kg bw/day (1.1 mg Ni/kg bw/day)** in this study (EU-RAR 2008b).

In the 3-generation reproduction study Wistar rats were administered 0, 250, 500, or 1000 ppm nickel (**nickel sulphate hexahydrate**) in the diet (EU-RAR 2008b; see above). The number of pups born dead was increased at all nickel doses in the F1a generation and at 500 ppm and 1000 ppm in the F1b generation, but there was no effect on pup mortality in later generations. There was a clear and consistent decrease averaging 27% in mean weanling body weight at 1000 ppm in all generations. The study authors state that there was no evidence of teratogenicity, based on gross examinations, and no histopathologic effects on the F3b generation. Evaluation of this study is complicated by the lack of statistical analyses and the reporting of results using pups rather than litters as the unit. Statistical analysis of the number of pups born dead show that the increased numbers at all doses levels in F1a and at 500 and 1000 ppm in F1b is statistically significant. Consequently the LOAEL in the study is set to the lowest dose level investigated, i.e. 250 ppm (13-20 mg Ni/kg bw/day).

Further studies on developmental toxicity are cited in the EU-RAR on nickel chloride (EU-RAR 2008d); since they do not contribute to evaluation they are not described.

Biomonitoring

Examination of nickel in blood and urine is in principle feasible for biological monitoring of nickel and nickel compounds. For occupational nickel exposure, a good correlation between nickel in plasma and urine was demonstrated with a relation of 8:1. Measurement of nickel in urine is favoured due to practical reasons. Background concentrations for nickel in urine are usually below 1 µg/l and may reach 3 µg/l. The following correlations between nickel concentrations in the air and nickel concentrations in urine were derived (Drexler and Greim, 1994; 2004):

| Air Water soluble nickel (mg/m ³) | Urine Nickel (µg/l) |
|---|------------------------|
| 0.025 | 25 |
| 0.050 | 40 |
| 0.100 | 70 |

| Air Poorly soluble nickel, metallic nickel (mg/m ³) | Urine Nickel (µg/l) |
|---|------------------------|
| 0.1 | 15 |
| 0.3 | 30 |
| 0.5 | 45 |

Based on these correlations and linear extrapolation, a concentration of 0.01 mg/m^3 corresponds to nickel concentrations in urine of $10 \text{ } \mu\text{g/l}$ in case of readily soluble nickel compounds. In case of poorly water soluble nickel compounds nickel concentrations in urine are lower due to the long persistence of nickel in the lung. Thus, a concentration of 0.01 mg/m^3 would correspond to $1.5 \text{ } \mu\text{g/l}$ in case of metallic and poorly soluble nickel compounds after linear extrapolation from higher exposure levels. Since this lies within the urinary background concentration, significant increases should be avoided.

Recommendation

Exposure to nickel compounds is associated with an increased cancer risk in the lung and nasal cavity, as well as with inflammatory responses/fibrosis in the lung.

The proposed OEL is based on protection from inflammatory effects in the lung, but according to available evidence should also protect against carcinogenic effects.

Concerning inflammatory responses, the inhalation study in rats with nickel sulfate showed a pronounced inflammatory reaction at a concentration of 0.06 mg/Ni/m^3 whereas no inflammatory effects were observed in rats at 0.03 mg Ni/m^3 , which is regarded as the NOAEL. The other available long-term inhalation studies with other nickel species in rats do not allow identification of NOAELs. In case of poorly soluble nickel compounds, pronounced inflammatory reactions including fibrosis were seen at 0.11 mg Ni/m^3 for nickel subsulfide and 0.5 mg Ni/m^3 for nickel oxide. The inhalation study with metallic nickel revealed alveolar proteinosis, alveolar histiocytosis and chronic inflammation at the lowest concentration of 0.1 mg/m^3 .

From the NOAEL of 0.027 mg/m^3 rounded to 0.03 mg/m^3 for the water-soluble nickel sulfate an 8 hr OEL of 0.01 mg/m^3 is recommended. This value most likely also protects from the inflammatory effects of insoluble nickel compounds and metallic nickel.

This value should also protect against nickel-induced carcinogenicity, from chromosomal damage and from indirect genotoxicity.

The carcinogenicity of nickel compounds has been clearly demonstrated in epidemiological studies. Within the different cohorts attempts have been made to rank the carcinogenic potentials for the different nickel species, water soluble nickel, particulate nickel compounds and metallic nickel. Both water soluble and poorly water soluble, particulate nickel compounds are to be considered as carcinogenic in humans, whereas epidemiological studies on metallic nickel do not indicate a carcinogenic potential. However, epidemiological data alone are not considered sufficient to exclude any nickel species such as metallic nickel from further considerations, since there are no cohorts that have been exclusively exposed to one nickel species. With respect to animal studies in rats, mice or hamsters, long-term inhalation studies revealed carcinogenicity in the lung and nasal cavity in case of poorly soluble nickel compounds (nickel oxide: 1.0 mg Ni/m^3 ; nickel subsulfide: $> 0.11 \text{ mg Ni/m}^3$), but not in one inhalation study

with water soluble nickel compounds. The latter observation appears to contradict the carcinogenic activity of water soluble nickel compounds in humans, and may be due to the high toxicity and resulting limitations in exposure concentrations. Metallic nickel caused malignant tumors after intratracheal instillation and intraperitoneal injection in rats, but no significant increase in lung tumors was observed in a recently conducted inhalation study.

From a mechanistic point of view, nickel and nickel compounds are not directly mutagenic, but have clastogenic activity. Based on cellular investigations, at low concentrations nickel ions do not directly interact with DNA but rather exert indirect genotoxic effects such as interference with DNA repair systems and DNA methylation patterns, which lead to an increased genomic instability. These effects are mediated by nickel ions, even though it cannot be excluded that on conditions of particle overload chronic inflammation may contribute to the carcinogenicity (see mode of action).

With respect to quantitative estimates on the carcinogenicity in humans, the International Committee on Nickel Carcinogenicity in Man (ICNCM, 1990) concluded that the increase in cancers of the nasal cavity (ethmoid) and lungs (bronchi, etc.) among workers in nickel refineries is associated with a minimum exposure of 1 mg/m^3 for water soluble salts and 10 mg/m^3 for insoluble compounds (sulfides, oxide, etc.) of nickel. However, a Finnish epidemiological study (Antilla, 1998) revealed an excess of bronchial cancer and two cancers of the sinuses (nasal cavity) among workers exposed to concentrations of about 0.25 mg/m^3 water soluble nickel salts (sulfate). Concerning the Kristiansand cohort, a significant increase in cancer incidence for water soluble nickel was observed at a cumulative exposure of $1.6 \text{ mg/m}^3 \times \text{years}$, equivalent to 0.04 mg Ni/m^3 when calculated for 40 years exposure (Grimsrud et al., 2002). However, this would resemble a worst case estimate, since current evidence strongly suggests indirect mechanisms with sublinear dose-response relationships in the low concentration range.

Increased frequencies of chromosomal aberrations in humans were observed at exposure levels above 0.5 mg/m^3 . Nickel levels in plasma and urine at the proposed TWA of 0.01 mg/m^3 would be around $80 \text{ } \mu\text{g/l}$ and $10 \text{ } \mu\text{g/l}$, respectively, corresponding to 1.4 or $0.17 \text{ } \mu\text{M}$, which is below DNA repair inhibitory concentrations in experimental systems in vitro. Nickel concentrations in the lung would be expected to be comparable to plasma levels, i.e. in the low μM range.

Due to the bioavailability of all nickel compounds, biomonitoring of nickel especially after exposure towards water-soluble nickel is indicated. The upper limit of background urinary concentration is $3 \text{ } \mu\text{g/l}$. Based on the correlations between nickel concentrations in the air and nickel in urine, a concentration of 0.01 mg/m^3 corresponds to nickel concentrations of $10 \text{ } \mu\text{g/l}$ urine for water soluble nickel and to $1.5 \text{ } \mu\text{g/l}$ urine for poorly soluble nickel compounds. Since the latter value lies within the background level of nickel in urine, any elevation should be avoided.

The reproductive system is also regarded as a potential target for the inorganic compounds of nickel, both in animal experiments and in humans. Exposure to nickel sulphate and nickel chloride in multi-generation studies and in the one-generation studies provide consistent evidence of developmental toxicity (stillbirth, postimplantation/perinatal death) in rats at dose levels not causing maternal toxicity. Based on the increased postimplantation/perinatal lethality

in the F1 generation in the two-generation study with **nickel sulphate**) at 2.2 mg Ni/kg bw/day (EU-RAR 2008b) and the marginal increase in pup mortality in the one-generation study with **nickel chloride** at 1.33 mg Ni/kg bw/day (EU-RAR 2008d), the calculated NOAEL used in the EU Risk Assessment Report for risk characterisation was 1.1 mg Ni/kg bw/day (EU-RAR 2008b). Assuming 10% oral absorption, 70 kg bw and 10 m³ inhaled air in 8 hours, the NOAEL of 1.1 mg Ni/kg bw/day is equivalent to 0.77 mg Ni/m³.

Nickel and nickel compounds can cause contact dermatitis, contact urticaria, allergic rhinitis and allergic asthma. The proposed OEL of 0.01 mg Ni/m³ does not take into account the sensitizing effects of nickel and nickel compounds.

In summary, the proposed OEL is based on the protection from non-cancer-effects of the lung. Taken into account the indirect mode of action of nickel-induced carcinogenicity available evidence suggests that it will also prevent carcinogenicity. Also, this OEL is about 70-fold lower than the NOAEL for reproductive toxicity.

No measurement difficulties are foreseen neither at the airborne nor at the biological levels recommended.

References

- Aberer W, Holub H (1992) Berufsdermatologische Relevanz der Nickelsensibilisierung. (Occupational dermatological relevance of nickel sensitization) (German). *Allergologie* 15: 429–432
- Andersen A, Svenes KB (1989) Determination of nickel in lung specimens of thirty-nine autopsied nickel workers. *Int Arch Occup Environ Health* 61: 289–295
- Andersen A, Berge SR, Engeland A, Norseth T (1996) Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. *Occup Environ Med* 53: 708–713
- Angerer J, Heinrich-Ramm R, Lehnert G (1989) Occupational exposure to cobalt and nickel. Biological monitoring. *Int J Environ Anal Chem* 35: 81–88
- Anttila A, Pukkala E, Aitio A, Rantanen T, Karjalainen S (1998) Update of cancer incidence among workers at a copper/nickel smelter and nickel refinery. *Int Arch Occup Environ Health* 71: 245–250
- Arena VC, Sussman NB, Redmond CK, Costantino JP, Trauth JM (1998) Using alternative comparison populations to assess occupation-related mortality risk. *J Occup Environ Med* 40: 907–916
- Bal W, Lukszo J, Kasprzak KS (1996) Interactions of nickel(II) with histones: enhancement of 2'-deoxyguanosine oxidation by Ni(II) complexes with CH₃CO-Cys-Ala-Ile-His-NH₂, a putative metal binding sequence of histone H3. *Chem Res Toxicol* 9: 535–540
- Benson, J. M., Burt, D. G., Carpenter, R. I., *et al.* (1988) Comparative inhalation toxicity of nickel sulfate to F344/N rats and B6C3F1 mice exposed for twelve days. *Fundam Appl Toxicol* 10, 164–171
- Block GT, Yeung M (1982) Asthma induced by nickel. *J Am Med Assoc* 247: 1600–1602

- Broday L, Cai J, Costa M (1999) Nickel enhances telomeric silencing in *Saccharomyces cerevisiae*. *Mutat Res* 440: 121–130
- Broday L, Peng W, Kuo MH, Salnikow K, Zoroddu M, Costa M (2000) Nickel compounds are novel inhibitors of histone H4 acetylation. *Cancer Res* 60: 238–241
- Chakrabarti, S.K., Bai, C., Subramanian, KS. (2001) DNA-protein crosslinks induced by nickel compounds in isolated rat lymphocytes: role of reactive oxygen species and specific amino acids. *Toxicol. Appl. Pharmacol.* 170, 153-165
- Chang J, Watson WP, Randerath E, Randerath K (1993) Bulky DNA adduct formation induced by nickel(II) *in vitro* as assayed by 32P-postlabelling. *Mutat Res* 291: 147–159
- Chorvatovicova D (1983) The effect of NiCl₂ on the level of chromosome aberrations in Chinese hamster *Cricetulus griseus*. *Biológia (Bratislava)* 38: 1107–1112
- Cicarelli RB, Wetterhahn KE (1984) Nickel-bound chromatin, nucleic acids, and nuclear proteins from kidney and liver of rats treated with nickel carbonate *in vivo*. *Cancer Res* 44: 3892–3897
- Cirla AM, Baruffini A, Pisati G, Zedda S (1982) Allergic bronchial reactions due to stainless steel welding fumes. *Lav Um* 30: 17–20
- Costa M (1996) Mechanisms of nickel genotoxicity and carcinogenicity. in: Chang LW (Ed.) Toxicology of metals, CRC Press, Boca Raton, 245–251
- Costa M, Mollenhauer HH (1980) Phagocytosis of nickel subsulfide particles during the early stages of neoplastic transformation in tissue culture. *Cancer Res* 40: 2688–2694
- Costa M, Klein CB (1999) Nickel carcinogenesis, mutation, epigenetics, or selection. *Environ Health Perspect* 107: A438–A439
- Cronin E (1980) Contact dermatitis. Churchill Livingstone, Edinburgh, 338–366
- Dally H, Hartwig A (1997) Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. *Carcinogenesis* 18: 1021–1026
- Davies JE (1986) Occupational asthma caused by nickel salts. *J Soc Occup Med* 36: 29–30
- Deknadt G, Leonard A (1982) Mutagenicity tests with nickel salts in the male mouse. *Toxicology* 25: 289–292
- Deng CZ, Lee HCH, Xian HL, Yao MC, Huang JC, Ou BX (1988) Chromosomal aberrations and sister chromatid exchanges of peripheral blood lymphocytes in Chinese electroplating workers: effect of nickel and chromium. *J Trace Elem Exp Med* 1: 57–62
- Dhir H, Agharwal K, Sharma A, Talukder G (1991) Modifying role of *Phyllanthus emblica* and ascorbic acid against nickel clastogenicity in mice. *Cancer Lett* 59: 9–18
- Doll R (1990) Report of the international committee on nickel carcinogenesis in man. *Scand J Work Environ Health* 16: 1–82
- Dolovich J, Evans SL, Nieboer E (1984) Occupational asthma from nickel sensitivity: I. Human serum albumin in the antigenic determinant. *Br J Ind Med* 41: 51–55
- Drexler H, Greim H (2004) Nickel (leichtlösliche Nickelverbindungen wie Nickelacetat und vergleichbare lösliche Salze, Nickelchlorid, Nickelhydroxid, Nickelsulfat). 12. Lieferung 2004, Wiley-VCH, Weinheim
- Dunnick, J. K., Elwell, M. R., Benson, J. M., *et al.* (1989) Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate in F344/N rats and B6C3F1 mice. *Fundam Appl Toxicol* 12, 584–594
- Dunnick JK, Elwell MR, Radovsky AE, Benson JM, Hahn FF, Nikula KJ, Barr EB, Hobbs CH (1995) Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. *Cancer Res* 55: 5251–5256

- Easton DF, Peto J, Morgan LG, *et al.* (1992) Respiratory cancer mortality in Welsh nickel refiners: which nickel compounds are responsible? In: Niebor E, Nrigau JO (Ed) Nickel and Human Health: Current Perspectives, Wiley & Sons, New York, 603–619
- Enterline PE, Marsh GM (1982) Mortality among workers in a nickel refinery and alloy manufacturing plant in West Virginia. *J Nat Cancer Inst* 68: 925–933
- Estlander T, Kanerva L, Tupasela O, Keskinen H, Jolanki R (1993) Immediate and delayed allergy to nickel with contact urticaria, rhinitis, asthma and contact dermatitis. *Clin Exp Allergy* 23: 306–310
- The ESSCA Writing Group. The European Surveillance System of Contact Allergies (ESSCA): results of patch testing the standard series, 2004. *JEADV* 2008; 22: 174-181.
- EU-RAR (EU Risk Assessment Report) (2008 a) Nickel. Danish Environmental Protection Agency, Copenhagen
- EU-RAR (EU Risk Assessment Report) (2008 b) Nickel and nickel compounds. Danish Environmental Protection Agency, Copenhagen
- EU-RAR (EU Risk Assessment Report) (2008 c) Nickel sulphate. Danish Environmental Protection Agency, Copenhagen
- EU-RAR (EU Risk Assessment Report) (2008 d) Nickel chloride. Danish Environmental Protection Agency, Copenhagen
- EU-RAR (EU Risk Assessment Report) (2008 e) Nickel carbonate. Danish Environmental Protection Agency, Copenhagen
- EU-RAR (EU Risk Assessment Report) (2008 f) Nickel dinitrate. Danish Environmental Protection Agency, Copenhagen
- Grandjean P (1984) Human exposure to nickel. Nickel in the human environment. *IARC Sci Publ* 53: 469–485
- Greim H (ed) (2006) Nickel and its compounds (in the form of inhalable dusts/aerosols). MAK Value Documentations, Volume 22, The MAK-Collection for Occupational Health and Safety, Wiley-VCH, Weinheim
- Greim H, Borm P, Schins R, Donaldson K, Driscoll K, Hartwig A, Kuempel E., Oberdörster G, Speit G (2001) Toxicology of Fibers and Particles-Report of the Workshop Held in Munich, Germany, October 26-27, 2000. *Inhal Toxicol* 13: 737–754
- Grimsrud TK, Peto J (2006) Persisting risk of nickel related lung cancer and nasal cancer among Clydach refiners. *Occup Environ Med* 63: 365–366
- Grimsrud TK, Berge SR, Haldorsen T, Andersen A (2002) Exposure to different forms of nickel and risk of lung cancer. *Am J Epidemiol* 156: 1123–1132
- Groth DH, Stettler LE, Burg JR, Busey WM, Grant GC, Wong L (1986) Carcinogenic effects of antimony trioxide and antimony ore concentrate in rats. *J Toxicol Environ Health* 18: 607–626
- Haber LT, Erdreich L, Diamond GL, Maier AM, Ratney R, Zhao Q, Dourson ML. Hazard identification and dose response of inhaled nickel-soluble salts (2000). *Regul Toxicol Pharmacol*, 31:210–230
- Hamdan S, Morse B, Reinhold D (1999) Nickel subsulfide is similar to potassium dichromate in protecting normal human fibroblasts from the mutagenic effects of benzo[a]pyrene diol epoxide. *Environ Mol Mutagen* 33: 211–218
- Hannu T, Piipari R, Tuppurainen M, Nordman H, Tuomi T (2007) Occupational asthma caused by stainless steel welding fumes: a clinical study. *Eur Respir J* 29: 85-90
- Harnett PB, Robison SH, Swartzendruber DE, Costa M (1982) Comparison of protein, RNA and DNA binding, and cell-cycle-specific growth inhibitory effects of nickel compounds in cultured cells. *Toxicol Appl Pharmacol* 64: 20–30

- Hartwig H (ed) (2008) *Gesundheitsschädliche Arbeitsstoffe – Toxikologisch medizinische Begründungen von MAK-Werten*, 45. Lieferung, Nickel und Nickelverbindungen, Wiley-VCH, Weinheim
- Hartwig A, Mullenders LH, Schlepegrell R, Kasten U, Beyersmann D (1994) Nickel(II) interferes with the incision step in nucleotide excision repair in mammalian cells. *Cancer Res* 54: 4045–4051
- Hsieh TH, Yu CP, Oberdörster G (1999a) A dosimetry model of nickel compounds in the rat lung. *Inhalat Toxicol* 11: 229–248
- Hsieh TH, Yu CP, Oberdörster G (1999b) Modeling of deposition and clearance of inhaled Ni compounds in the human lung. *Regul Toxicol Pharmacol* 30: 18–28
- IARC (International Agency for Research on Cancer) (1990) Chromium, nickel and welding. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans, Volume 49, IARC, Lyon 257–445
- ICNCM (International Committee on Nickel Carcinogenicity in Man) (1990) Report of the International Committee on Nickel Carcinogenesis in Man. *Scand J Work Environ Health* 16, 1–84
- ICPS (1991) Environmental health Criteria, 108, Nickel, WHO.
- Ishimatsu S, Kawamoto T, Matsuno K, Kodama Y (1995) Distribution of various nickel compounds in rat organs after oral administration. *Biol Trace Elem Res* 49: 43–52
- Iwitzki F, Schlepegrell R, Eichhorn U, Kaina B, Beyersmann D, Hartwig A (1998) Nickel(II) inhibits the repair of O6-methylguanine in mammalian cells. *Arch Toxicol* 72: 681–689
- Johansson A, Camner P, Jarstrand C, Wiernik A (1980) Morphology and function of alveolar macrophages after long-term nickel exposure. *Environ Res* 23: 170–180
- Karjalainen S, Kerttula R, Pukkala E (1992) Cancer risk among workers at a copper/nickel smelter and nickel refinery in Finland. *Int Arch Occup Environ Health* 63: 547–551
- Kasprzak KS, Hernandez L (1989) Enhancement of hydroxylation and deglycosylation of 2'-deoxyguanosine by carcinogenic nickel compounds. *Cancer Res* 49: 5964–5968
- Kasprzak KS, Diwan BA, Konishi N, Misra M, Rice JM (1990) Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. *Carcinogenesis* 11: 647–652
- Kasprzak KS, Jaruga P, Zastawny TH, North SL, Riggs CW, Olinski R, Dizdaroglu M (1997) Oxidative DNA base damage and its repair in kidneys and livers of nickel(II)-treated male F344 rats. *Carcinogenesis* 18: 271–277
- Katsifis SP, Shamy M, Kinney LP, Burns FJ (1998) Interaction of nickel with UV-light in the induction of cytogenetic effects in human peripheral lymphocytes. *Mutat Res* 422: 331–337
- Kawanishi S, Oikawa S, Inoue S, Nishino K (2002) Distinct Mechanisms of Oxidative DNA Damage Induced by Carcinogenic Nickel Subsulfide and Nickel Oxides. *Environ Health Perspect* 110: 789–791
- Keskinen H, Kalliomäki PL, Alanko K (1980) Occupational asthma due to stainless steel welding fumes. *Clin Allergy* 10: 151–159
- Kiilunen M, Utela J, Rantanen T, Norppa H, Tossavainen A, Koponen M, Paakkulainen H, Aitio A (1997) Exposure to soluble nickel in electrolytic nickel refining. *Ann Occup Hyg* 41: 167 – 188
- Kligman A (1966) The identification of contact allergens by human assay. III. The maximisation test: a procedure for screening and rating contact sensitizers. *J Invest Dermatol* 47: 393–409
- Kopera, E., T. Schwerdtle, A. Hartwig, W. Bal (2004) Co(II) and Cd(II) substitute for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity, *Chem. Res. Toxicol.*, 17, 1452 – 1458.
- Krueger I, Mullenders LHF, Hartwig A (1999) Nickel(II) increases the sensitivity of V79 Chinese hamster cells towards cisplatin and transplatin by interference with distinct steps of DNA repair. *Carcinogenesis* 20: 1177–1184

- Lee JE, Cicarelli RB, Wetterhahn KJ (1982) Solubilization of the carcinogen nickel subsulfide and its interaction with deoxyribonucleic acid and protein. *Biochemistry* 21: 771–778
- Lee KP, Trochimowicz HJ, Reinhardt CF (1985) Pulmonary response of rats exposed to titanium dioxide (TiO₂) by inhalation for two years. *Toxicol Appl Pharmacol* 79: 179–192
- Lee SH, Shiao Y-H, Plisov SY, Kasprzak KS (1999) Nickel(II) acetate-treated Chinese hamster ovary cells differentially express vimentin, hSNF2H homologue, and H ferritin. *Biochem Biophys Res Commun* 258: 592–595
- Lee Y-W, Klein CB, Kargacin B, Salnikow K, Kitahara J, Dowjat K, Zhitkovich A, Christie NT, Costa M (1995) Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol Cell Biol* 15: 2547–2557
- Lee Y-W, Broday L, Costa M (1998) Effects of nickel on DNA methyltransferase activity and genomic DNA methylation levels. *Mutat Res* 415: 213–218
- Lin X, Sugiyama M, Costa M (1991) Differences in the effect of vitamin E on nickel sulfide or nickel chloride-induction of chromosomal aberrations in mammalian cells. *Mutat Res* 260: 159–164
- Lloyd DR, Philips DH (1999) Oxidative DNA damage mediated by copper(II), iron(II) and nickel(II) Fenton reactions: evidence for site-specific mechanisms in the formation of doublestrand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. *Mutat Res* 424: 23–36
- Malo JL, Cartier A, Doepner M, Nieboer E, Evans S, Dolovich J (1982) Occupational asthma caused by nickel sulfate. *J Allergy Clin Immunol* 69: 55–59
- Mathur AK, Dikshith TSS, Lal MM, Tandon SK (1978) Distribution of nickel and cytogenetic changes in poisoned rats. *Toxicology* 10: 105–113
- Mauderly JL. Contribution of Inhalation Bioassay to the Assessment of Human Health Risk from Solid Airborne Particles. In: Mohr, U., Dungworth, D.L., Mauderly, J.L., Oberdörster, G. (eds): Toxic and Carcinogenic Effects of Solid Particles. Washington, ILSI Press, pp 355-365 (1994)
- Mayer C, Klein RG, Wesch H, Schmezer P (1998) Nickel subsulfide is genotoxic *in vitro* but shows no mutagenic potential in respiratory tract tissues of BigBlue™ rats and Muta™ Mouse mice *in vivo* after inhalation. *Mutat Res* 420: 85–98
- Menné T, Calvin G (1993) Concentration threshold of non-occluded nickel exposure in nickelsensitive individuals and controls with and without surfactant. *Contact Dermatitis* 29: 180–184
- Mohanty PK (1987) Cytotoxic effect of nickel chloride on the somatic chromosomes of Swiss albino mice *mus musculus*. *Curr Sci* 56: 1154–1157
- Mollerup S, Rivedal E, Moehle L, Haugen A (1996) Nickel(II) induces alterations in EGF- and TGF-β₁-mediated growth control during malignant transformation of human kidney epithelial cells. *Carcinogenesis* 17: 361–367
- Morita H, Umeda M, Ogawa HI (1991) Mutagenicity of various chemicals including nickel and cobalt compounds in mouse FM3A cells. *Mutat Res* 261: 131–137
- Morita T, Asano N, Awogi T, Sasaki YF, Sato S-I, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS-MMS. *Mutat Res* 389: 3–122
- Muhle H, Bellmann B, Takenaka S, Fuhst R, Mohr U, Pott F (1990) Chronic effects of intratracheally instilled nickel-containing particles in hamsters. in: Nieboer E, Nriagu JO (Eds) Nickel and human health: current perspectives, John Wiley and Sons, New York
- Nackerdien Z, Kasprzak KS, Rao G, Halliwell B, Dizdaroglu M (1991) Nickel(II)- and cobalt(II)-dependent damage by hydrogen peroxide to the DNA bases in isolated human chromatin. *Cancer Res* 51: 5837–5842

- Nielsen GD, Rohold AE, Andersen KE (1992) Nickel contact sensitivity in the guinea pig. An efficient open application test method. *Acta Derm Venereol* 72: 45–48
- Norseth T (1986) Nickel. in: Friberg L, Nordberg GF, Vouk V (Eds) Handbook on the toxicology of metals, Volume 2, 2nd edition, Elsevier, Amsterdam, 462–481
- NTP (National Toxicology Program) (1993) Toxicology and carcinogenesis studies of talc in F344/N rats and B6C3F1 mice. Technical report series No. 421, NIH No 93-3152, Research Triangle Park, NC, USA
- NTP (1996 a) Toxicology and carcinogenesis studies of nickel oxide in F344/N rats and B6C3F1 mice. Technical report series No. 451, US Department of Health and Human Services, Washington, DC, USA
- NTP (1996 b) Toxicology and carcinogenesis studies of nickel subsulfide in F344/N rats and B6C3F1 mice. Technical report series No. 453, US Department of Health and Human Services, Washington, DC, USA
- NTP (1996 c) Toxicology and carcinogenesis studies of nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Technical report series No. 454, US Department of Health and Human Services, Washington, DC, USA
- Oller AR, Costa M, Oberdörster G (1997) Carcinogenicity assessment of selected nickel compounds. *Toxicol Appl Pharmacol* 143: 152–166
- Oller AR, Erexson G (2007) Lack of micronuclei formation in bone marrow of rats after repeated oral exposure to nickel sulfate hexahydrate. *Mutat Res* 626:102–110
- Oller AR, Kirkpatrick DT, Ann Radovsky R, Hudson K, Bates HK (2008) Inhalation carcinogenicity study with nickel metal powder in Wistar rats. *Toxicol Appl Pharmacol*, article in press
- Osmundsen PE (1980) Contact urticaria from nickel and plastic adhesives (butylhydroxytoluene, oleylamide). *Contact Dermatitis* 6: 452–455
- Ottolenghi AD, Haseman JK, Payne WW, Falk HL, MacFarland HN (1974) Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. *J Natl Cancer Inst* 54: 1165–1172
- Pang D, Burges DCL, Sorahan T (1996) Mortality study of nickel platers with special reference to cancers of the stomach and the lung, 1945–93. *Occup Environ Med* 53: 714–717
- Porter DW, Yakushiji H, Nakabeppu Y, Sekiguchi M, Fivash MJ, Kasprzak KS (1997) Sensitivity of *Escherichia coli* (MutT) and human (MTH1) 8-oxo-dGTPase to *in vitro* inhibition by the carcinogenic metals, nickel(II), copper (II), cobalt (II) and cadmium (II). *Carcinogenesis* 18: 1785–1791
- Pott F, Ziem U, Reiffer F-J, Huth F, Ernst H, Mohr U (1987) Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. *Exp Pathol (Jena)* 32: 129–152
- Pott F, Rippe RM, Roller M, Rosenbruch M, Huth F (1990) Vergleichende Untersuchungen über die Kanzerogenität verschiedener Nickelverbindungen und Nickellegierungen (Comparative investigations into the carcinogenicity of various nickel compounds and nickel alloys) (German), Wirtschaftsverlag NW, Bremerhaven
- Pott F, Rippe RM, Roller M, Csicsaky M, Rosenbruch M, Huth F (1992) Carcinogenicity of nickel compounds and nickel alloys in rats by intraperitoneal injection. in: Nieboer E, Nriagu JO (Eds) Nickel and human health: current perspectives, John Wiley and Sons, New York
- Raithel HJ (1987) Forschungsbericht Nickel (Research report on nickel) (German), Schriftenreihe des Hauptverbandes der gewerblichen Berufsgenossenschaften e. V., Sankt Augustin
- Redmond CK (1984) Site-specific cancer mortality among workers involved in the production of high nickel alloys. in: Sunderman FW Jr (Ed.) Nickel in the human environment, IARC, Lyon, *IARC Sci Publ* 53: 73–86

- Salnikow K, Cosentino S, Klein C, Costa M (1994) Loss of thrombospondin transcriptional activity in nickel-transformed cells. *Mol Cell Biol* 14: 851–858
- Salnikow K, Blagosklonny MV, Ryan H, Johnson R, Costa M (2000) Carcinogenic nickel induces genes involved with hypoxic stress. *Cancer Res* 60: 38–41
- Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM, Kasprzak KS (2004) Depletion of Intracellular Ascorbate by the Carcinogenic Metals Nickel and Cobalt Results in the Induction of Hypoxic Stress. *J Biol Chem* 279: 40337–40344
- Schnuch A, Uter W, Lehmacher W, Fuchs T, Enders F, Arnold R, Bahmer F, Brasch J, Diepgen TL, Frosch PJ, Henseler T, Müller S, Peters KP, Schulze-Dirks A, Sary A, Zimmermann J (1993) Epikutantestung mit der Standardserie. Erste Ergebnisse des Projektes "Informationsverbund Dermatologischer Kliniken" (IVDK) (Epicutaneous testing with the standard series. First results of the "Informationsverbund Dermatologischer Kliniken" (IVDK) project) (German). *Dermatosen Beruf Umwelt* 41: 60–70
- Schnuch A, Geier J, Lessmann H, Uter W (2003) Rückgang der Nickelkontaktallergie in den letzten Jahren. Eine Folge der „Nickel-Verordnung“? Auswertungen der Daten des IVDK der Jahre 1992–2001 (Decrease in nickel sensitization in young patients – successful intervention through nickel exposure regulation? Results of the IVDK, 1992–2001) (German). *Hautarzt* 54: 626–632
- Schwerdtle, T., A. Seidel and A. Hartwig (2002) Effect of soluble and particulate nickel compounds on the formation and repair of stable benzo[a]pyrene DNA adducts in human lung cells. *Carcinogenesis*, 23, 47 – 53.
- Schwerdtle, T. and A. Hartwig (2006), Bioavailability and genotoxicity of soluble and particulate nickel compounds in cultured human lung cells. *Materials Science and Engineering Technology*, 37, 521-525.
- Seilkop SK (1997) Stellungnahme zu der Arbeit von Andersen *et al.* (1996) für NiPERA (Nickel Producers Environmental Research Association) (Statement on the study of Andersen *et al.* (1996) for NiPERA (Nickel Producers Environmental Research Association) (German)
- Senft V, Losan F, Tucek M (1992) Cytogenetic analysis of chromosomal aberrations of peripheral lymphocytes in workers occupationally exposed to nickel. *Mutat Res* 279: 171–179
- Shirakawa T, Kusaka Y, Fujimura N, Kato M, Heki S, Morimoto K (1990) Hard metal asthma: cross immunological and respiratory reactivity between cobalt and nickel? *Thorax* 45: 267–271
- Shirakawa T, Kusaka Y, Morimoto K (1992) Specific IgE antibodies to nickel in workers with known reactivity to cobalt. *Clin Exp Allergy* 22: 213–218
- Smith, M.K., George, E.L., Stober, J.A., Feng, H.A., Kimmel, G.L. (1993) Perinatal toxicity associated with nickel chloride exposure. *Environ. Res.* 61: 200 – 211.
- Sunderman Jr FW (1984) Carcinogenicity of nickel compounds in animals. *IARC Sci Publ* 53: 127–142
- Sunderman Jr FW, Aitio A, Morgan LG, Norseth T (1986) Biological monitoring of nickel. *Toxicol Ind Health* 2: 17–78
- Sunderman Jr FW, Hopfer SM, Sweeney KR, Marcus AH, Most BM, Creason J (1989) Nickel absorption and kinetics in human volunteers. *Proc Soc Exp Biol Med* 191: 5–11
- Svenes KB, Andersen I (1998) Distribution of nickel in lungs from former nickel workers. *Int Arch Occup Environ Health* 71: 424–428
- Torreilles J, Guerin M-C, Slaoui-Hasnaoui A (1990) Nickel(II) complexes of histidyl-peptides as Fenton-reaction catalysts. *Free Radical Res Commun* 11: 159–166
- Vandenberg JJ, Epstein WL (1963) Experimental contact sensitization in man. *J Invest Dermatol* 44: 413–416
- Wahlberg JE (1989) Nickel: animal sensitization assays. in: Maibach HI, Menné T (Eds) Nickel and the skin: immunology and toxicology, CRC Press Inc, Florida, 65–73

- Waksvik H, Boysen M (1982) Cytogenetic analyses of lymphocytes from workers in a nickel refinery. *Mutat Res* 103: 185–190
- Waksvik H, Boysen M, Hogetveit AC (1984) Increased incidence of chromosomal aberrations in peripheral lymphocytes of retired nickel workers. *Carcinogenesis* 5: 1525–1527
- Weischer CH, Kordel W, Hochrainer D (1980) Effects of NiCl₂ and NiO in Wistar rats after oral uptake and inhalation exposure respectively. *Zbl Bakt Hyg, 1. Abt. Orig B.* 171, 336–351
- Wozniak, K., Blasiak, J. (2002) Free radicals-mediated induction of oxidized DNA bases and DNA-protein cross-links by nickel chloride. *Mutation Research* 514, 233 – 243
- Zhou D, Salnikow K, Costa M (1998) Cap43, a novel gene specifically induced by Ni²⁺ compounds. *Cancer Res* 58: 2182–2189