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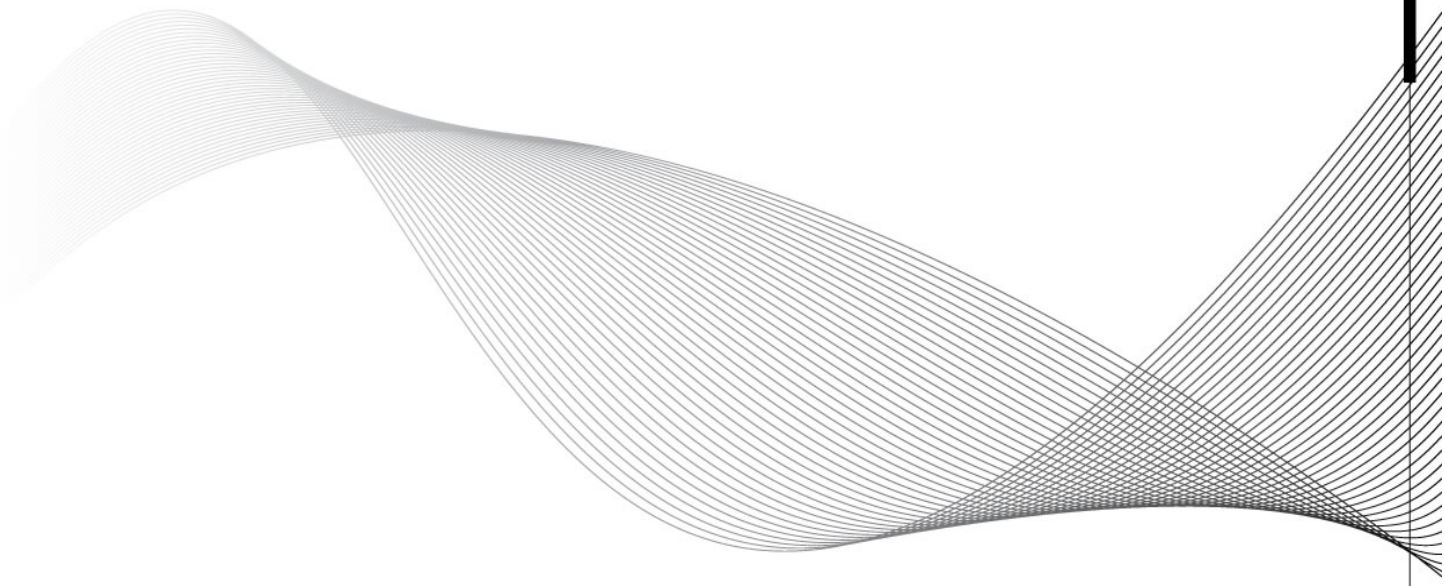

Recommendation from the Scientific Committee on Occupational Exposure Limits for nickel and inorganic nickel compounds

SCOEL/SUM/85

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8 hour TWA	: 0.005 mg Ni/m³ 0.01 mg Ni/m³	(respirable fraction) (inhalable fraction, excluding metallic nickel)
STEL (15 min)	: --	
Notation	: --	
Biological Guidance value (BGV)	: 3 µg/L urine	
SCOEL carcinogen group	: C	(carcinogen with a practical threshold) (excluding metallic nickel)

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	Xn & R40
Nickel monoxide, nickel(II) oxide	NiO	1313-99-1	215-215-7	74.69	poorly soluble	T & R49, R43
Nickel dioxide, nickel(IV) oxide	NiO_2	12035-36-8	234-823-3	90.69	poorly soluble	T & R49, R43
Dinickel trioxide, nickel trioxide, nickel(III) oxide	Ni_2O_3	1314-06-3	215-217-8	165.38	poorly soluble	T & R49, R43
Nickel hydroxide	$Ni(OH_2)$	12054-48-7	235-008-5	92.72	0.013	Xn & R20/22, R40, R43
Nickel carbonate	$NiCO_3$	3333-67-3	222-068-2	118.70	0.0093 [25°C]	Xn & R20, R40, R43
Nickel sulphide	NiS	16812-54-7	240-841-2	90.77	0.00036 [18°C]	T & R49, R43
Nickel subsulphide	Ni_3S_2	12035-72-2	234-829-6	240.26	poorly soluble	T & R49, R43
Nickel	$NiCl_2$	7718-54-9	231-743-0	129.61	254 [20°C]	T & R45, R25,

Substance, synonyms	Molecular formula	CAS Number	EINECS Number	Molecular weight	Solubility in water (g/100 ml) [temperature]	Classification
chloride						R36/38, R43
Nickel nitrate	$Ni(NO_3)_2$	13138-45-9	236-068-5	182.72	238.5 [0°C]	T & R45, R22, R43
Nickel sulphate	$NiSO_4$	7786-81-4	232-104-9	154.77	65.5 [0°C]	Xn & R22, R40, R42/43
Nickel acetate	$Ni(CH_3CO_2)_2$	373-02-4	206-761-7	176.80	17 [20°C]	T & R45, R22; R43

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 (100 – 250 µg per day), drinking water (up to 20 µg per day) and cigarettes (4 – 8 µg per 20 cigarettes). Nickel intake from inhalative exposure to ambient air is rather low (about 0.04 µg/m^3 in cities). The bioavailability from oral intake is low (about 2%), while absorption from ambient air or cigarettes is nearly 100 or 50%, respectively (summarized in IPCS, 1991 and EU RAR, 2008 b).

1. Occurrence/ use and occupational exposure

Nickel is a hard, silvery-white metal. It is very widely distributed in mining, in the heavy industries (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 sulphides 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 sulphidic 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 sulphides. 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). Mathematical modelling of the deposition and clearance of inhaled nickel compounds in rats and humans also showed that the lung retention times in rats and humans are substantially longer for the poorly soluble compounds nickel monoxide and nickel subsulphide than for the readily soluble nickel sulphate (Hsieh *et al.* 1999 a, b). A retention time similar to poorly soluble nickel compounds was found for metallic nickel in an inhalation study with Wistar rats (NIPERA, 2008). Concerning the systemic **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 readily soluble or poorly soluble nickel compounds including metallic nickel, indicating the systemic 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 intracellular release of nickel ions due to gradual dissolution of nickel particles in the lysosomes (Costa and Mollenhauer 1980, Johansson *et al.* 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 phagocytozed by alveolar macrophages (Johansson *et al.* 1980).

The systemic 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 sulphate, 9.8% for nickel chloride, 0.47% for nickel subsulphide, 0.09% for nickel metal, and 0.01% for nickel oxide (Haber et al. 2000). Nickel ingested orally accumulated particularly in the kidneys (Ishimatsu *et al.* 1995).

2.2. Acute Toxicity

Nickel at high doses and in certain forms is toxic to both man and animals. For example, the oral LD_{50} of nickel acetate was 350 mg/kg bw in rats and 420 mg/kg bw in mice. In case of nickel chloride intraperitoneal LD_{50} 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

2.4.1. 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-releasing 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

The occurrence of nickel-induced asthma among exposed workers is rare compared to contact dermatitis (Fernández-Nieto et al. 2006). The incidence of occupational asthma among stainless steel welders in Finland was 0.9-2 per 1000 workers each year (Hannu et al 2007). In the literature, there have been several case reports of nickel-induced asthma associated with exposure to nickel sulphate; some have been confirmed by inhalation challenge test. Specific IgE antibodies to nickel-human serum albumin conjugate have been reported in some cases (Dolovich et al. 1984; Estlander et al 1993; Fernández-Nieto et al. 2006; Kusaka et al. 1991; Nieboer et al. 1984). Nevertheless, there are actually only few case reports suggesting evidence for specific IgE, positive skin tests and positive provocation tests with nickel sulphate in exposed persons, and pointing to a workplace related asthmatic diseases (Bright et al. 1997; Cirla et al. 1982). Recently, lymphocyte transformation was demonstrated in patients with nickel-induced asthma, suggesting that cell-mediated hypersensitivity may play a part in nickel induced asthma (see Cruz et al. 2006). However, in most occupational activities, salts of – mostly transition – metals and nickel are usually manipulated in combination (Fernández-Nieto et al. 2006). In some cases, cell-mediated immunity to nickel as well as cobalt is implicated with hard metal asthma (Kusaka et al. 1991).

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).

2.4.2. 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 sulphate 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 sulphate, 7 of 12 to 1% and 4 of 12 to 3% nickel sulphate. Irritation to the skin was induced more often with lanolin than with 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 sulphate hexahydrate aerosol at concentrations of 0, 0.12, 0.25, or 0.5 mg compound/m^3 (0, 0.027, 0.056, or 0.11 mg Ni/m^3) for 6 h/day, 5 days/week for 2 years. At the 2-year sacrifice, non-neoplastic inflammatory lesions of the lung were observed at exposure concentrations $\geq$ 0.056 mg Ni/m^3 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/m^3 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/m^3) was not statistically significant following exposure for 15 months or 2 years. A definitive conclusion regarding the adversity of the endpoint is not possible. 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/m^3.** The NOAEL for atrophy of the olfactory epithelium was 0.056 mg Ni/m^3 (Haber *et al.* 2000).

Two further chronic inhalation studies were performed by NTP. In one study, male and female rats were exposed to nickel subsulphide at concentrations of 0, 0.15, or 1 mg compound/m^3 (0, 0.11, 0,73 mg Ni/m^3) for 6 h/day, 5 days/week for 2 years. Even at the lowest 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 compond/m^3 (0, 0.5, 1.0, 2.0 mg Ni/m^3). 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/m^3 (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/m^3, most pronounced in female rats. Mean body weights in the 0.4 mg/m^3 group males and females were 27% and 18% lower than controls, respectively. In the 0.1 mg/m^3 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/m³)										
		Nickel sulphate hexahydrate				Nickel subsulphide			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****				
Interstitium, 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-neplastic histopathological lung lesions in rats in 2-year inhalation studies with nickel metal (Oller et al. 2008)

Selected lesions	Males			Females		
	Concentration (mg Ni/m³)					
	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

[a] Incidence based on 50 animals per group, except in Group 3 (0.4 mg Ni/m³) that had 54 females;
[b] Chronic inflammation includes both chronic and chronic-active inflammation;
[c] Incidence based on the following number of animals per group: 34 and 39 for Group

In a chronic study in the mouse (NTP 1996 c), males and females 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

2.6.1. Effects in humans

Main effects are summarized in Table 2. There was a higher incidence of metaphases with gaps, but no or only not significantly increased frequencies of sister chromatid exchanges in lymphocytes of workers 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 workers exposed to **sulphidic** 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/m³ 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/m³ 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/m³, >25 years exposed (8 years after retirement)	Increased gaps and breaks; no increased SCE frequency	Waksvik *et al.* 1984

Industry	Exposure	Results	References
Electroplating with nickel (n=7)	0.005-0.094 mg Ni/m³	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 *et al.* 1988
NiO production (n=6)	mean : 0.77 mg/m³ (range : 0.28 – 1.52)	9.5% chromosome aberrations as compared to 4.05%	Senft *et al.* 1992
$NiSO_4$ production (n=15)	mean: 1.3 mg/m³ (range: 0.31 – 2.86)	5.2% chromosome aberrations as compared to 4.05%	Senft *et al.* 1992
Control group (n=19)	control group later recognised as also exposed to nickel	4.05% chromosome aberrations (normal value ≤2%)	Senft *et al.* 1992
Electrolysis (n=25)	230-800 µg Ni/m³ workplace air; 0.9-2.4 µg Ni/m³ behind face mask	no increased number of micronuclei in buccal mucous cells	Kiilunen *et al.* 1997

2.6.2. 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 sulphide**, c**rystalline nickel subsulphide** and **nickel oxide** caused morphological cell transformation in Syrian hamster embryo cells. Crystalline nickel sulphide and crystalline nickel subsulphide 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 subsulphide 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 the repair 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 sulphate	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 sulphide	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)	

Substance	Test system	Result (lowest effective dose in µg Ni/ml)	References
	V79 hamster cells	mutagenic in hprt gene (4.9)	
Amorphous nickel sulphide	Syrian hamster embryo cells	no morphological transformation	IARC 1990
	CHO cells	no DNA breaks	
Crystaline nickel subsulphide	Syrian hamster embryo cells	morphological transformation (3.7)	IARC 1990
	CHO cells	weakly mutagenic in hprt gene (1.1)	
	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_3S_2/l)	Mayer et al. 1998

In vivo

The genotoxic properties of nickel compounds *in vivo* - performed in most cases with intraperitoneal application - are shown in Table 4.

Nickel chloride caused chromosome aberrations in the bone marrow of mice (≥3 mg Ni/kg bw; Mohanty 1987; 5 mg Ni/kg bw; Dhir *et al.* 1991) and Chinese hamsters (≥1.25 mg Ni/kg bw; Chorvatovicova 1983).). Conflicting results were obtained for the induction of micronuclei in the bone marrow of mice. While nickel chloride was positive in one study (5 mg Ni/kg bw; Dhir *et al.* 1991), no micronuclei were found for **nickel chloride** in another study with lower doses **(**1.6 mg Ni/kg bw; Morita *et al.* 1997**)**, with **nickel nitrate** (12 mg Ni/kg bw; Deknut and Leonard 1982), **nickel sulphate** (8 mg Ni/kg bw; Morita *et al*. 1997; 112 mg Ni/kg bw; Oller and Erexson 2007) or **nickel oxide** (114 mg Ni/kg bw; Morita *et al.* 1997). **Nickel chloride** and **nickel nitrate** were negative in the dominant lethal test in mice (11 and 12 mg Ni/kg bw; Deknudt and Leonard 1982). **Nickel carbonate** induced DNA breaks and crosslinks in kidney but not in liver (5 mg Ni/kg bw; Ciccarelli and Wetterhahn 1984), and **nickel acetate** induced oxidative DNA damage in kidney and liver (5 mg Ni/kg bw; Kasprzak *et al.* 1997). **Nickel subsulphide** was not mutagenic in the respiratory tract of transgenic rats or mice (3 mg Ni/kg bw; Mayer *et al.* 1998).

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

Substance	Species (route of administration)	Dose (mg/kg bw)	Highest ineffective/ lowest effective dose (mg Ni/kg bw)	Effect	References
Nickel chloride	mouse (i.p.)	6–24 (3–12 mg Ni/kg bw)	3	increased chromosome aberrations in bone marrow cells	Mohanty 1987
	Chinese hamster (i.p.)	2–10 (4–20% LD_{50}) (1.25–5 mg Ni/kg bw)	1.25	increased chromosome aberrations in bone marrow cells	Chorvatori cova 1983
	mouse (i.p.)	25 (11 mg Ni/kg bw)	11	negative dominant lethal test, negative micronucleus test	Deknudt and Leonard 1982
	mouse (i.p.)	10–40 (1 x) (5–20 mg Ni/kg bw)	5 (6, 12, 24 h)	increased chromosome aberrations in bone marrow cells	Dhir *et al.* 1991
	mouse (i.p.)	10–40 (2 x) (5–20 mg Ni/kg bw)	5 (24, 48 h)	induction of micronuclei	Dhir *et al.* 1991
	mouse (i.p.)	3.2–25 (1.6–12.5 mg Ni/kg bw)	12.5	negative micronucleus test	Morita et al. 1997
Nickel nitrate	mouse (i.p.)	56 (12 mg Ni/kg bw)	12	negative dominant lethal test, negative micronucleus test	Deknudt and Leonard 1982
Nickel monoxide	mouse (i.p.)	18–145 (14–114 mg Ni/kg bw)	114	negative micronucleus test	Morita *et al.* 1997
Nickel carbonate	rat (i.p.)	10–40 (5–20 mg Ni/kg bw)	5	DNA breaks and DNA protein cross links in kidney, but not in liver	Ciccarelli and Wetterhahn 1984
Nickel sulphate	mouse (i.p.)	5–20 (2–8 mg Ni/kg bw)	8	negative micronucleus test	Morita *et al.* 1997
	rat (i.p.)	35– 70 (3–6 mg Ni/kg bw; 9–14 d)	6	no increased chromosome aberrations in bone marrow cell or testes	Mathur *et al.* 1978

Substance	Species (route of administration)	Dose (mg/kg bw)	Highest ineffective/ lowest effective dose (mg Ni/kg bw)	Effect	References
	rat (oral)	125, 250, 500 (28, 56, 112 mg Ni/kg bw; 3 d; nickel sulphate hexahydrate)	112 (absorbed dose: 5.6–32 mg Ni/kg bw)	negative micronucleus test	Oller and Erexson 2007
Nickel acetate	mouse (i.p.)	30 (10 mg Ni/kg bw)	10	DNA modifications (^{32}P-postlabelling)	Chang *et al.* 1993
	mouse (i.p.)	16 (5 mg Ni/kg bw)	5	oxidative DNA damage in liver and kidneys	Kasprzak *et al.* 1997
Nickel subsulphide	transgene rat	4–13 (MTD) (1–3 mg Ni/kg bw) nose-only-inhalation	3	no mutagenicity in the lacI gene	Mayer *et al.* 1998
	transgenic mouse		3	no mutagenicity in the lacI gene	

2.7. Carcinogenicity

2.7.1. Effects in humans

Carcinogenic effects of nickel have long been recognized. The main target is the respiratory system and tumours 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, and, although procedures changed and exposure levels dropped, a modestly increased lung cancer risk has persisted at a relatively constant level (SMR ≈ 140) in workers hired after 1930 (summarized in Grimsrud and Peto, 2006). The reported high smoking prevalence in these workers may have contributed to the cancer risk (Sorahan and Williams, 2005). Based on evidence of increased respiratory cancer risk of cohorts of workers from different countries engaged in refining and processing of sulphidic nickel ores, 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 sulphide 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 (≥5 mg/m^3, or ≥10 mg/m^3) were recorded among employees working in the linear calciners and cleaning reducers in the nickel plant, where there was a high exposure to oxidic and sulphidic nickel at the same time. While 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, statistical analyses of cumulative exposure data showed no compelling evidence of metallic nickel inducing these cancers. The same could not be said for various other nickel compounds, which showed evidence of an association of exposure to excess nasal and lung cancer risks. Moreover, 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 (or below 2 mg/m^3 as inhalable nickel) (Doll 1990, IARC 1990).

Additional information on metallic nickel in the producing industry can be gleaned from the latest update of a study of 1,649 hydrometallurgical refinery workers in Canada (Egedhal *et al.,* 2001). The number of workers exposed to nickel was small (718), but the exposures in this plant were solely to nickel concentrates and metallic nickel. Exposures to metallic nickel were high, with a mean of 6 mg Ni/m^3 (as inhalable). No excess risks of lung cancer were found in these workers (Relative Risks, RR=0.67; 95% Confidence Interval, CI, 0.24-1.46); nor were any deaths due to nasal cancers detected.

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 reported by Arena *et al.* (1998) 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. These exposure data were largely based upon measurements taken from one plant in the late-1970s. A recent reconstruction of historical exposures of these alloy workers (Sivulka and Seilkop, 2009) indicated that average exposures in process areas outside of powder metallurgy ranged between ≈0.3 and 1.8 mg Ni/m^3, with an overall average of 0.65 mg Ni/m^3 (1.5 mg/m^3 as inhalable). 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 there was no evidence of a duration of exposure-based response in lung cancer deaths when comparisons were made with the U.S. population, 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 – in order to better control for factors that were not occupationally-related. 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 was 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 (or 0.4 mg/m³ as inhalable) (compared to $\geq$ 1 mg/m³ or > 1.6 mg/m³ as inhalable in Kristiansand electrolysis workers). 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 leaching, calcining, and 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 the processing of sulphidic nickel matte, which included work areas such as roasting and smelting, with relatively high insoluble nickel exposures (>5 mg Ni/m³, or >11.5 mg Ni/m³ as inhalable) and lower levels of soluble nickel exposure, as well as electrolytic nickel refining using sulphuric acid and were exposed mainly to high concentrations (≥ 1 mg/m³ or >1.6 mg Ni/m³ as inhalable) of nickel sulphate and much lower concentrations of other, poorly soluble nickel compounds. The highest lung cancer risks occurred in electrolysis workers and the highest nasal cancer risks appeared to be in roasting and smelting workers (Doll, 1990). 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 authors also indicated that there was an interaction for lung cancer between smoking and exposure to total nickel. The lung cancer risk of Kristiansand workers has been further analysed in a case control study with diagnoses occurring 1952-1995 (Grimsrud et al., 2002). This study indicated that lung cancer risk appeared more strongly associated with soluble nickel exposure than with exposure to other nickel compounds, although soluble and insoluble nickel exposures were highly correlated. In the past, particularly high exposures to soluble nickel occurred in the electrolysis department (Doll 1990). The data suggesting a role for soluble nickel in the carcinogenic process still seem to be convincing, but effects due to 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 cumulative exposure to soluble nickel[a] (data from Andersen *et al.* 1996)

Exposure range (mg Ni/m³) x years	Mean exposure (mg Ni/m³) x years	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

An update (Grimsrud *et al.,* 2003) confirmed the main findings in earlier reports of the increased lung cancer risk among Norwegian workers. The analyses demonstarted an association between risk and length of employment or duration of nickel exposure. For the Norwegian cohort a strong association was observed for cumulative exposure to water-

soluble nickel and lung cancer risk, when nickel exposure, historical exposure to arsenic, cobalt, asbestos and acid mists were considered (Grimsrud *et al.,* 2005).

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 (SIR 41.1; 95% CI 4.97–148) 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. 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 (Antilla *et al.,* 1998).

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.

Sulphidic and oxidic nickel compounds

It is difficult to differentiate between sulphidic and oxidic compounds in epidemiology since sulphides are generally calcined to oxides in nickel-producing plants. Altogether, there were increased risks of lung and nasal cancers among persons exposed to sulphidic and oxidic nickel. One example is the Clydach cohort. It consisted of 2521 men employed in various processes in the refining of nickel from grinding and roasting of nickel containing sulphidic 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 sulphidic, oxidic and metallic nickel dusts and, to a lower extent, to soluble nickel salts in a hydrometalurgical 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 mainly to changes in the feed material, but also to the introduction of gauze masks in some areas of the refinery, 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 sulphidic nickel being a carcinogen: Among workers with relatively low exposure to oxidic and soluble nickel, but high exposure to sulphidic nickel, the lung cancer risk was clearly higher than among those

with lower exposure to sulphidic 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, 2003, 2005), the Clydach cohort (South Wales) (Easton et al., 1992) and the Harjavalta cohort (Finnland) (Antilla et al., 1998). Concerning the water insoluble nickel species, reevaluation of the Clydach cohort revealed that insoluble nickel species (oxidic, sulphidic or metallic) contributed to cancer risk (Easton et al., 1992). A general contribution, albeit not dose-dependent, of sulphidic 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 sulphate induced no tumours in rats or mice (Dunnick *et al.* 1995, NTP 1996c).

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.

2.7.2. 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 combined adenomas and carcinoma of the adrenal cortex in female rats (Oller et al. 2008; see Table 7). 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 tumours in rats exposed to nickel metal by inhalation (Oller et al. 2008)

	Concentration (mg Ni/m³)					
	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. 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 sulphate): 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 an oral carcinogenicity study no increased tumour incidence was observed (Heim *et al.,* 2007). In the only published inhalation study with nickel sulphate 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 sulphate were, however, limited to 0.11 mg/m^3 in rats and 0.22 mg/m^3 in mice, since toxicity (pneumonia) occurred at higher doses (in comparison: According to the results of studies of exposed workers, the carcinogenic airborne concentrations of soluble nickel were 0.25 mg/m^3 and higher).

Conclusion: The only animal inhalation study with a soluble nickel salt (nickel sulphate) as well as an oral study with this salt yielded negative results. This seems to contradict epidemiological studies where soluble nickel compounds appear to at least contribute to nickel-induced carcinogenicity.

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).

Sulphidic nickel: Crystalline, but not amorphous nickel monosulphide 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 subsulphide 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 subsulphide 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 (MMAD 1.8 µm; GSD 2.4 µm)	Controls 0.1 mg/m³ 0.4 mg/m³	0/50 5/50 21/50*	benign and malignant pheochromocytoma	Oller et al. 2008
Wistar rat, female	inhalative, 6h/d, 5d/w, 2 years (MMAD 1.8 µm; GSD 2.4 µm)	Controls 0.1 mg/m³ 0.4 mg/m³	2/50 2/49 **7/54***	adenoma and carcinoma of the adrenal cortex	Oller et al. 2008
Wistar rat, female	intratracheal, 2.5 years	Controls 20 x 0.36 mg Ni/rat, once weekly 10 x 0.9 mg Ni/rat, once weekly	0/40 **10/39** **8/32**	malignant tumours	Pott *et al.* 1987
Wistar rat, female	i.p., 2 years	Controls 1 x 6 mg Ni/rat 2 x 6 mg Ni/rat 25 x 1 mg Ni/rat	4/133 **4/34*** **5/34*** **25/35**	local malignant tumours	Pott *et al.* 1992
Syrian hamster, male, female	intratracheal, 26 - 30 month	Controls 12 x 0.8 mg Ni/ hamster at 14 day intervals	0/60 1/60	adenocarcinoma	Muhle *et al.* 1990
Nickel acetate					
Wistar rat, female	i.p., 2 years	Controls 25 x 1 mg Ni/rat, once weekly 50 x 1 mg Ni/rat, twice weekly	1/33 3/35 **5/31***	malignant tumours	Pott *et al.* 1992
Nickel sulphate (hexahydrate)					
Fischer rat, female	inhalative, 6h/d, 5d/w, 2 years (MMAD 2.5 µm; GSD 2.38 µm)	Controls 0.11 mg Ni/m³ (MTD)	0/52 1/54	adenoma (NTP: "no evidence")	NTP 1996 c
Fischer rat, male	inhalative, 6h/d, 5d/w, 2 years (MMAD 2.5 µm; GSD 2.38 µm)	Controls 0.11 mg Ni/m³ (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/m³ (MTD)	7/61 2/60	adenoma + carcinoma (NTP: "no evidence")	NTP 1996 c

Species, sex	Route of administration, study duration	Dose	Tumour incidence	Type of tumour[1)]	References
B6C3F1 mouse, male	inhalative, 6h/d, 5d/w, 2 years	Controls 0.22 mg Ni/m³ (MTD)	13/61 8/61	adenoma + carcinoma (NTP: "no evidence")	NTP 1996 c
Fischer rat, males and females	oral (gavage), 2 years	Controls 10, 30 or 50 mg/kg bw		no evidence of tumours	Heim *et al.*, 2007
Nickel(II) oxide					
Fischer rat, female	inhalative, 6h/d, 5d/w, 2 years (MMAD 2.21 µm; GSD 1.97 µm)	Controls 0.5 mg Ni/m³ 1.0 mg Ni/m³ 2.0 mg Ni/m³	1/53 0/53 6/53 (p=0.056) 5/64	adenoma + carcinoma (NTP: "**some evidence**")	NTP 1996 a
Fischer rat, male	inhalative, 6h/d, 5d/w, 2 years (MMAD 2.21 µm; GSD 1.97 µm)	Controls 0.5 mg Ni/m³ 1.0 mg Ni/m³ 2.0 mg Ni/m³	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/m³ 2.0 mg Ni/m³ 3.9 mg Ni/m³	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/m³ 2 mg Ni/m³ 3.9 mg Ni/m³	9/57 14/67 15/66 14/69	adenoma + carcinoma (NTP: "no evidence")	NTP 1996 a
Nickel sulphide					
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
Nickel subsulphide					
Fischer rat, female	inhalative, 6 h/d, 5d/w, 80 weeks	Controls 0.73 mg Ni/m³	1/107 **12/98****	adenoma + carcinoma	Ottolenghi *et al.* 1974
Fischer rat, male	inhalative, 6 h/d, 5d/w, 80 weeks	Controls 0.73 mg Ni/m³	1/108 **17/110****	adenoma + carcinoma + sarcoma	Ottolenghi *et al.* 1974
Fischer rat, female	inhalative, 6 h/d, 5d/w, 2 years (MMAD 2.17 µm; GSD 2.34 µm)	Controls 0.11 mg Ni/m³ 0.73 mg Ni/m³	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 (MMAD 2.17 µm; GSD 2.34 µm)	Controls 0.11 mg Ni/m³ 0.73 mg Ni/m³	0/53 **6/53*** **11/53****	adenoma + carcinoma (NTP: "**clear evidence**")	NTP 1996 b
B6C3F1 mouse,	inhalative, 6 h/d, 5d/w,	Controls 0.44 mg Ni/m³	9/58 2/59	adenoma + carcinoma	NTP 1996 b

Species, sex	Route of administration, study duration	Dose	Tumour incidence	Type of tumour[1)]	References
female	2 years	0.88 mg Ni/m³	3/60	(NTP: "no evidence")	
B6C3F1 mouse, male	inhalative, 6 h/d, 5d/w, 2 years	Controls	13/61	adenoma + carcinoma	NTP 1996 b
		0.44 mg Ni/m³	5/59		
		0.88 mg Ni/m³	6/58	(NTP: "no evidence")	

[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 presence of nickel ions at target cellular sites is responsible for the inflammatory, genotoxic and/or carcinogenic effects of nickel compounds. Additionally, the long half live of insoluble particles may contribute to adverse effects in the lung due to the longer retention time and thus longer time for interactions with target cells.

The 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, sulphidic 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 phagocytozed by alveolar macrophages of exposed rabbits (Johansson *et al.* 1980). Nickel subsulphide was phagocytozed *in vitro* by CHO cells (Costa and Mollenhauer 1980; 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 subsulphide, 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 human soluble nickel compounds appear to be stronger carcinogens as compared to less soluble and metallic species. This discrepancy

may be due to the high toxicity of readily soluble nickel compounds in animals, which did not allow the application of concentrations relevant for human exposure. However, also problems with the interpretation of epidemiological data may contribute here.

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 subsulphide 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 Ni_3S_2, NiO or $NiSO_4$. 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 tumour 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 O^6-methylguanine-methyltransferase (MGMT) repairing almost exclusively O^6-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 tumour 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/m^3 (Dunnick *et al.* 1995), lung tumours were induced at 18 mg/m^3 for talcum (NTP 1993) and at 45 mg/m^3 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/m^3, 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.

2.8. 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/m^3 as **nickel sulphate 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/m^3 (rats) or 0.22 mg Ni/m^3 (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/m^3 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 histopatholgy 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). As a limited number of animals per group was used a clear NOAEL for fertility 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, and higher dose levels than 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) need to be examined.

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 Fla 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 are uncertainties concerning this NOAEL.

Developmental toxicity

Human Data

In several studies the reproductive health of a large group of female nickel refinery workers exposed to water-soluble nickel species was investigated. There was no increased risk for newborns with genital malformations or undescendend testis (Vaktskjold *et al.*, 2006), for newborns small-for-gestational-age (Vaktskjold *et al.*, 2007) or for newborns with musculoskeletal defects (22,965 births; Vaktskjold *et al.*, 2008 a). In a case-control study, the adjusted odds ratio for spontaneous abortions was slightly but statistically non

significantly increased (OR 1.14; 95% confidence interval 0.95-1.37; Vaktskjold *et al.*, 2008 b).

Data with Experimental Animals

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/m^3 (0.6, 1.2, or 2.5 mg Ni/m^3) for 21 days, beginning on gestation day 1. Maternal endpoints evaluated were body weight, organ weights, serum urea, and haematology. The only foetal endpoints evaluated were foetal weight, leukocytes, and serum urea. Maternal body weight gain was statistically significantly reduced in all exposed groups, and statistically significant decreases in foetal body weight were observed at the top two exposure levels. Foetal weight was significantly decreased at the mid- and high-concentration level. Other developmental effects, such as foetal 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. Measurement of nickel in urine is favoured due to practical reasons, preferably after several consecutive working shifts. Background concentrations for nickel in urine of a reference population of working age are usually below 1 µg/l and may reach 3 µg/l (Hartwig and Drexler 2010). Based on 95. percentile values derived in non-occupationally exposed populations in several studies, a biological guidance value of 3 µg nickel/l urine is proposed.

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.

Since mechanistic data indicate an indirect genotoxic mode of action, nickel is considered a carcinogen group C (carcinogen with a practical threshold).

The proposed OELs are 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 sulphate showed a pronounced inflammatory reaction at a concentration of 0.06 mg/Ni/m^3 whereas no consistent inflammatory effects were observed in rats at 0.03 mg Ni/m^3, which is regarded as the NOAEL.

Starting from the NOAEL of 0.027 mg Ni/m^3 rounded to 0.03 mg/m^3 in case of water-soluble nickel sulphate derived in the NTP study, differences between rats and humans with respect to particle deposition in the alveolar region need to be considered. 0.03 mg Ni/m^3 in rats corresponds to an equivalent human concentration (EHC) of 0.016 mg/m^3 (Oller and Oberdoerster, 2010). Since this conversion only takes into account the deposited dose and not the long-term chronic retained dose as well as potential toxicodynamic differences, an 8 h OEL of 0.005 mg Ni/m^3 is proposed. Considering the particle size of nickel sulphate of 2.5 µm mass median aerodynamic diameter (MMDA) applied in the NTP study, the proposed value corresponds to the respirable fraction.

The available long-term inhalation studies with other nickel species in rats do not allow the identification of NOAELs. In case of poorly water soluble nickel compounds, pronounced inflammatory reactions including fibrosis were seen at 0.11 mg Ni/m^3 for nickel subsulphide and 0.5 mg Ni/m^3 for nickel oxide, respectively. The inhalation study with metallic nickel revealed alveolar proteinosis, alveolar histocytosis and chronic inflammation at the lowest concentration of 0.1 mg/m^3. In all three cases this was the lowest concentration applied. Due to the severe lung damage observed at these concentrations (fibrosis in almost all

(Ni_3S_2) or all animals (NiO), or chronic inflammation in basically all rats (metallic nickel), respectively, the 2-3-fold higher deposition of nickel after exposure to nickel oxide in humans as compared in rats (Oller and Oberdoerster, 2010) as well as the estimated far longer retention half-times in humans as compared to rats for Ni_3S_2 and NiO (Oller and Oberdoerster), **an OEL of 0.005 mg/m³ (respirable fraction) is proposed for poorly soluble nickel compounds and metallic nickel as well.**

In addition to chronic inflammation of the lung, the proposed OEL also needs to protect from nickel-induced carcinogenicity. Since epidemiological evidence suggests not only the induction of lung tumours, which may be provoked by respirable particle sizes, but also of nasal tumours, and particles at the workplace are not limited to the respirable fraction, exposure towards inhalable nickel particles needs to be limited for carcinogenic nickel species as well.

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. It has to be emphasized, however, that 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 in case of poorly soluble nickel compounds (nickel oxide: 1.0 mg Ni/m³; nickel subsulphide: > 0.11 mg Ni/m³), 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 tumours after intratracheal instillation and intraperitoneal injection in rats, but no significant increase in lung tumours was observed in a recently conducted inhalation study.

From a mechanistic point of view, nickel and nickel compounds are not directly mutagenic. 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 clastogenicity and 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 (ethmoïd) and lungs (bronchi, etc.) among workers in nickel

refineries is associated with a minimum exposure of 1 mg/m³ for water soluble salts and l0 mg/m³ for insoluble compounds (sulphides, 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/m³ water soluble nickel salts (sulphate). Concerning the Kristiansand cohort, a significant increase in cancer incidence for water soluble nickel was observed at a cumulative exposure of 1.6 mg/m³ x years, equivalent to 0.04 mg Ni/m³ when calculated for 40 years exposure (Grimsrud et al., 2002). However, this would resemble a conservative estimate, since current evidence strongly suggests indirect mechanisms with sublinear dose-response relationships in the low concentration range.

Therefore, to protect from nickel-induced carcinogenicity, **an OEL of 0.01 mg Ni/m³ is proposed for the inhalable fraction of water soluble as well as poorly water soluble nickel compounds. Metallic nickel is excluded,** since neither animal data nor epidemiological data point towards a carcinogenic action of nickel metal. This value should also protect against nickel-induced indirect genotoxicity, including chromosomal damage. Thus, increased frequencies of chromosomal aberrations in humans were observed at exposure levels above 0.5 mg/m³. Nickel levels in plasma and urine at the proposed TWA of 0.01 mg/m³ would be around 80 µg/l and 10 µg/l, respectively, corresponding to 1.4 or 0.17 µM, 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.

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, which is well above the proposed OELs.

Nickel and nickel compounds can cause contact dermatitis and contact urticaria. Nickel is the most commonly diagnosed cause of allergic contact dermatitis worldwide. In contrast, cases of occupational asthma by exposure to nickel are rare and co-exposure e.g. to chromium, cobalt or hard metals usually occurred, indicating that nickel is not a significant respiratory sensitizer. Therefore the database does not contradict derivation of an OEL.

In summary, the proposed OELs are based on the protection from non-cancer-effects in the lung (chronic inflammation), but should also protect from nickel-induced carcinogenicity, taking into account the indirect mode of action of nickel-induced genotoxicity. Also, the OEL for the inhalable fraction is about 70-fold lower than the NOAEL for reproductive toxicity.

Exposure to nickel and nickel salts at workplaces might evoke contact sensitization and – in rare cases – also sensitization of the respiratory tract; these effects are not taken into account by setting the OEL.

A method to measure nickel in the air is available using atomic absorption spectrometry. The detection limits are given with 0.3 µg/m^3 (stationary sampling, air sample volume 45 l) and
3 µg/m^3 (personal sampling, air sample volume 0.42 l) (Kettrup and Greim 2003).

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