3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

Experimental studies on animals exposed to nickel and various nickel compounds were reviewed previously in the *IARC Monographs* (IARC, 1976, 1987). Recent reviews on the biological and carcinogenic properties of nickel have been compiled by Fairhurst and Illing (1987), Kasprzak (1987) and Sunderman (1989), among others. In addition, a detailed document on the health effects of nickel has been prepared for the Ontario (Canada) Ministry of Labour (Odense University, 1986). A comprehensive technical report on nickel, emphasizing mutagenicity and carcinogenicity, was published by the European Chemical Industry Ecology and Toxicology Centre (1989).

(*a*) *Metallic nickel and nickel alloys*

(i) *Inhalation*

Mouse: A group of 20 female C57Bl mice, two months of age, was exposed by inhalation to 15 mg/m^3 metallic nickel powder (> 99% pure; particle diameter, $\leq$4 μm) for 6 h per day on four or five days per week for 21 months. All mice had died by the end of the experiment. No lung tumour was observed. No control group was available (Hueper, 1958). [The Working Group noted the short duration of treatment.]

Rat: Groups of 50 male and 50 female Wistar rats and 60 female Bethesda black rats, two to three months of age, were exposed by inhalation to 15 mg/m^3 metallic nickel powder (> 99% pure nickel; particle diameter, $\leq$4 μm) for 6 h per day on four or five days per week for 21 months and observed up to 84 weeks. Histological examination of the lungs of 50 rats showed numerous multicentric, adenomatoid alveolar lesions and bronchial proliferations that were considered by the author as benign neoplasms. No specific control was included in the study (Hueper, 1958).

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[1]The Working Group was aware of studies in progress of the carcinogenicity of nickel, nickel acetate tetrahydrate, nickel alloys, nickel–aluminium alloys, nickel chloride hexahydrate, nickel oxide, nickel sulfide and nickel sulfate hexa- and heptahydrate in experimental animals by intraperitoneal, subcutaneous, inhalation and intratracheal administration (IARC, 1988b).

In a further experiment with Bethesda black rats, exposure to metallic nickel powder (99.95% nickel; particle diameter, 1-3 μm) was combined with 20-35 ppm (50-90 mg/m³) sulfur dioxide as a mucosal irritant; powdered chalk was added to prevent clumping. Exposure was for 5-6 h per day [nickel concentration unspecified]. Forty-six of 120 rats lived for longer than 18 months. No lung tumour was observed, but many rats developed squamous metaplasia and peribronchial adenomatoses (Hueper & Payne, 1962).

Guinea-pig: A group of 32 male and 10 female strain 13 guinea-pigs, about three months of age, was exposed by inhalation to 15 mg/m³ metallic nickel powder (>99% pure nickel) for 6 h per day on four or five days per week for 21 months. Mortality was high: only 23 animals survived to 12 months and all animals had died by 21 months. Almost all animals developed adenomatoid alveolar lesions and terminal bronchiolar proliferations. No such lesion was observed in nine controls. One treated guinea-pig had an anaplastic intra-alveolar carcinoma, and another had an apparent adenocarcinoma metastasis in an adrenal node, although the primary tumour was not identified (Hueper, 1958).

(ii) *Intratracheal instillation*

Rat: Two groups of female Wistar rats [number unspecified], 11 weeks of age, received either ten weekly intratracheal instillations of 0.9 mg metallic nickel powder [purity unspecified] or 20 weekly injections of 0.3 mg metallic nickel powder in 0.3 ml saline (total doses, 9 and 6 mg, respectively) and were observed for almost 2.5 years. Lung tumour incidence in the two groups was 8/32 (seven carcinomas, one mixed) and 10/39 (nine carcinomas, one adenoma), respectively; no lung tumour developed in 40 saline-treated controls maintained for up to 124 weeks. Pathological classification of the tumours in the two groups combined revealed one adenoma, four adenocarcinomas, 12 squamous-cell carcinomas and one mixed tumour. Average time to observation of the tumours was 120 weeks, the first tumour being observed after 98 weeks (Pott *et al.*, 1987).

Hamster: In a study reported in an abstract, groups of 100 Syrian golden hamsters received either a single intratracheal instillation of 10, 20 or 40 mg of metallic nickel powder (particle diameter, 3-8 μm) or of one of two nickel alloy powders (particle diameter, 0.5-2.5 μm; alloy I: 26.8% nickel, 16.2% chromium, 39.2% iron, 0.04% cobalt; alloy II: 66.5% nickel, 12.8% chromium, 6.5% iron, 0.2% cobalt) or four intratracheal instillations of 20 mg of one of the substances every six months (total dose, 80 mg). In the groups receiving single instillations of alloy II, the incidence of malignant intrathoracic tumours was reported as 1, 8 and 12%, respectively, suggesting a dose-response relationship. In the group receiving multiple instillations of alloy II, 10% of the animals developed intrathoracic malignant neoplasms, diagnosed as fibrosarcomas, mesotheliomas and rhabdomyosarcomas. Metallic

nickel induced comparable numbers and types of intrathoracic neoplasms, but no tumour was observed in animals treated with alloy I or in control animals (Ivankovic *et al.*, 1987).

A group of approximately 60 male and female Syrian golden hamsters (strain Cpb-ShGa 51), ten to 12 weeks of age, received 12 intratracheal instillations of 0.8 mg metallic nickel powder (99.9% nickel; mass median diameter, 3.1 μm) in 0.15 ml saline at two-week intervals (total dose, 9.6 mg). Additional groups were treated similarly with 12 intratracheal instillations of 3 mg pentlandite (containing 34.3% nickel; total dose, 36 mg), 3 or 9 mg chromium/nickel stainless-steel dust (containing 6.79% nickel; total doses, 36 and 108 mg) or 9 mg chromium stainless-steel dust (containing 0.5% nickel; total dose, 108 mg). The median lifespan was 90-130 weeks in the different groups. Two lung tumours were observed: an adenocarcinoma in the group that received nickel powder and an adenoma in the pentlandite-treated group. No lung tumour was observed in vehicle-treated controls or in the groups treated with stainless-steels (Muhle *et al.*, 1990). [The Working Group noted that no lung tumour was observed in the positive control group.]

(iii) *Intrapleural administration*

Rat: A group of 25 female Osborne-Mendel rats, six months of age, received injections of a 12.5% suspension of metallic nickel powder in 0.05 ml lanolin into the right pleural cavity [6.25 mg nickel powder] once a month for five months. A group of 70 rats received injections of lanolin only. The experiment was terminated after 16 months. Four of the 12 treated rats that were examined had developed round-cell and spindle-cell sarcomas at the site of injection; no control animal developed a local tumour [$p < 0.01$] (Hueper, 1952).

A group of five male and five female Fischer 344 rats, 14 weeks of age, received injections of 5 mg metallic nickel powder suspended in 0.2 ml saline into the pleura (total dose, 25 mg) once a month for five months. Two rats developed mesotheliomas within slightly over 100 days; no tumour occurred in 20 controls (Furst *et al.*, 1973). [The Working Group noted the limited reporting of the experiment.]

(iv) *Subcutaneous administration*

Rat: Groups of five male and five female Wistar rats, four to six weeks of age, received four subcutaneous implants of pellets (approximately 2×2 mm) of metallic nickel or nickel-gallium alloy (60% nickel) used for dental prostheses and were observed for 27 months. Local sarcomas were noted in 5/10 rats that received the metallic nickel and in 9/10 rats that received the nickel-gallium alloy. No local tumour occurred in ten groups of rats that received similar implants of other dental materials (Mitchell *et al.*, 1960).

(v) *Intramuscular administration*

Rat: A group of ten female hooded rats, two to three months of age, received a single intramuscular injection of 28.3 mg pure metallic nickel powder in 0.4 ml fowl serum into the right thigh. All animals developed rhabdomyosarcomas at the injection site within 41 weeks. Historical controls injected with fowl serum alone did not develop local tumours (Heath & Daniel, 1964).

Groups of 25 male and 25 female Fischer 344 rats [age unspecified] received five monthly intramuscular injections of 5 mg metallic nickel powder in 0.2 ml trioctanoin. Fibrosarcomas occurred in 38 treated animals but in none of a group of 25 male and 25 female controls given trioctanoin alone (Furst & Schlauder, 1971).

Two groups of ten male Fischer 344 rats, three months of age, received a single intramuscular injection of metallic nickel powder (3.6 or 14.4 mg/rat) in 0.5 ml penicillin G procaine. Surviving rats were killed 24 months after the injection. Sarcomas at the injection site were found in 0/10 and 2/9 treated rats, respectively, as compared with 0/20 vehicle controls (Sunderman & Maenza, 1976). [The Working Group noted the small number of animals used.]

Groups of 20 WAG rats [sex and age unspecified] received a single intramuscular injection of 20 mg metallic nickel powder in an oil vehicle [type unspecified]. A group of 56 control rats received 0.3 ml of the vehicle alone. Local sarcomas developed in 17/20 treated and 0/56 control rats (Berry *et al.*, 1984). [The Working Group noted the inadequate reporting of tumour induction.]

Groups of 20 or 16 male Fischer 344 rats, two to three months of age, received a single intramuscular injection of 14 mg metallic nickel powder (99.5% pure) or 14 mg (as nickel) of a ferronickel alloy ($NiFe_{1\text{-}6}$) in 0.3-0.5 ml penicillin G vehicle into the right thigh. Of the 20 rats receiving nickel powder, 13 developed tumours at the site of injection (mainly rhabdomyosarcomas), with an average latency of 34 weeks. No local tumour developed in the 16 rats given the ferronickel alloy, in 44 controls given penicillin G or in 40 controls given glycerol (Sunderman, 1984).

Groups of 40 male inbred WAG rats, 10-15 weeks of age, received a single intramuscular injection of 20 mg metallic nickel in paraffin oil. One group also received intramuscular injections of interferon at 5×10^4 U/rat twice a week beginning in the tenth week after nickel treatment. Rhabdomyosarcomas occurred in 14/30 and 5/10 rats in the two groups, respectively. Metallic nickel depressed natural killer cell activity. Prospective analysis of individual natural killer cell responses indicated that a persistent depression was restricted to rats that subsequently developed a tumour (Judde *et al.*, 1987).

Hamster: Furst and Schlauder (1971) compared the tumour response in Syrian hamsters with that of Fischer 344 rats (see above) to metallic nickel powder. Groups of 25 male and 25 female hamsters, three to four weeks old, received five

monthly intramuscular injections of 5 mg nickel powder in 0.2 ml trioctanoin. Two fibrosarcomas occurred in males. No local tumour occurred in 25 male and 25 female controls injected with trioctanoin alone.

(vi) *Intraperitoneal administration*

Rat: As reported in an abstract, a group of male and female Fischer rats [numbers unspecified], weighing 80-100 g, received intraperitoneal injections of 5 mg metallic nickel powder in 0.3 ml corn oil twice a month for eight months. A control group received injections of corn oil only. In the treated group, 30-50% of rats were reported to have developed intraperitoneal tumours (Furst & Cassetta, 1973).

A group of 50 female Wistar rats, 12 weeks of age, received ten weekly intraperitoneal injections of 7.5 mg metallic nickel powder [purity unspecified] (total dose, 75 mg). Abdominal tumours (sarcomas, mesotheliomas or carcinomas) developed in 46/48 (95.8%) rats at an average tumour latency of approximately eight months. Concurrent controls were not reported, but, in non-concurrent groups of saline controls, abdominal tumours were found in 0-6% of animals (Pott *et al.*, 1987).

Groups of female Wistar rats, 18 weeks of age, received single or repeated intraperitoneal injections of metallic nickel powder (100% nickel) or of one of three nickel alloys in 1 ml saline once or twice a week. All animals were sacrificed 30 months after the first injection. The incidences of local sarcomas and mesotheliomas in the peritoneal cavity are shown in Table 22. A dose-response trend was apparent for metallic nickel, and the tumour responses to the nickel alloys increased with the proportion of nickel present and the dose (Pott *et al.*, 1989, 1990). [The Working Group noted that the results at 30 months were available as an extended abstract only.]

(vii) *Intravenous administration*

Mouse: A group of 25 male C57Bl mice, six weeks old, received two intravenous injections of 0.05 ml of a 0.005% suspension of metallic nickel powder in 2.5% gelatin into the tail vein. Nineteen animals survived more than 52 weeks, and six survived over 60 weeks. No tumour was observed. No control group was used (Hueper, 1955). [The Working Group noted the short period of observation.]

Rat: A group of 25 Wistar rats [sex unspecified], 24 weeks of age, received intravenous injections of 0.5 ml/kg bw metallic nickel powder as a 0.5% suspension in saline into the saphenous vein once a week for six weeks. Seven rats developed sarcomas in the groin region along the injection route [probably from seepage at the time of treatment]. No control group was used (Hueper, 1955).

Table 22. Tumour responses of rats to intraperitoneal injection of nickel and nickel alloys[a]

Compound	Total dose (mg, as Ni)	Schedule	Mesotheliomas at two years	Sarcomas at two years	Local tumours at 30 months[b]
Metallic nickel	6	Single injection	3	0	4/34
	12	2 × 6 mg	3	2	5/34
	25	25 × 1 mg	16	9	25/35
Alloy (50% Ni)	50	Single injection	1	7	8/35
	150	3 × 50 mg	2	8	13/35
Alloy (29% Ni)[c]	50	Single injection	0	0	2/33
	100	2 × 50 mg	0	1	1/36
Alloy (66% Ni)[d]	50	Single injection	0	11	12/35
	150	3 × 50 mg	4	20	22/33
Saline		3 × 1 ml	0	1	1/33
		50 × 1 ml	0	0	0/34

[a]From Pott *et al.* (1989, 1990)
[b]Results not given separately for mesotheliomas and sarcomas
[c]Before milling: 32% Ni, 21% Cr, 0.8% Mn, 55% Fe
[d]Before milling: 74% Ni, 16% Cr, 7% Fe

(viii) *Intrarenal administration*

Rat: A group of 20 female Sprague-Dawley rats, weighing 120-140 g, received an injection of 5 mg metallic nickel in 0.05 ml glycerine into each pole of the right kidney. No renal carcinoma or erythrogenic response developed within the 12-month period of observation (Jasmin & Riopelle, 1976).

Groups of male Fischer 344 rats, approximately two months of age, received an intrarenal injection of 7 mg metallic nickel powder or of a ferronickel alloy ($NiFe_{1\cdot6}$; 7 mg Ni per rat) in 0.1 or 0.2 ml saline solution into each pole of the right kidney. The study was terminated after two years; the median survival time was 100 weeks in the two treated groups compared with 91 weeks in controls. Renal cancers occurred in 0/18 and 1/14 rats, respectively, compared with 0/46 saline-treated controls. The tumour was a nephroblastoma which was observed at 25 weeks (Sunderman *et al.*, 1984b).

(ix) *Implantation of ear-tags*

Rat: In a study carried out to assess the carcinogenicity of cadmium chloride, 168 male Wistar rats, six weeks of age, received identification ear-tags fabricated of nickel-copper alloy (65% Ni, 32% Cu, 1% Fe, 1% Mn). A total of 14 tumours, mostly osteosarcomas, developed within 104 weeks at the site of implantation. The authors

implicated nickel in the alloy as the probably causative agent and apparent local microbial infection as a contributory factor (Waalkes *et al.*, 1987).

(x) *Other routes of administration*

Rat: In groups of 20 WAG rats [sex and age unspecified] *subperiosteal injection* of 20 mg metallic nickel powder resulted in local tumours in 11/20 rats; *intramedullary injection* of 20 mg metallic nickel resulted in local tumours in 9/20 rats (Berry *et al.*, 1984). [The Working Group noted the absence of controls and the inadequate reporting of tumour induction.]

(xi) *Administration with known carcinogens*

Rat: Four groups of female Wistar rats [initial numbers unspecified], four to six weeks old, received intratracheal instillations of 1 or 5 mg 20-methylcholanthrene (MC) alone or with 10 mg metallic nickel powder (99.5% nickel). A fifth group received 10 mg metallic nickel powder only. At 12 weeks, squamous-cell carcinomas had developed as follows: 5 mg MC, 2/7; 5 mg MC plus Ni, 3/5; 1 mg MC, 0/8; 1 mg MC plus Ni, 0/7; metallic Ni alone, 0/7. Pretumorous lesions were more marked and the amount of epithelial metaplasia enhanced in groups receiving the combined treatment or MC only (Mukubo, 1978). [The Working Group noted the small number of animals used and the short duration of observation.]

(*b*) *Nickel oxides and hydroxides*

The compounds considered under this heading include a variety of substances of nominally similar composition, which, however, may vary considerably due to differences in production methods. These differences were not generally defined in the studies described below, beyond the relatively recent designation of green and black nickel oxide.

(i) *Inhalation*

Rat: Groups of six or eight male Wistar rats, two months of age, were exposed by inhalation to 0.6 or 8.0 mg/m^3 nickel monoxide (green) particles (median aerodynamic diameter, 1.2 μm) for 6 h per day on five days per week for one month, after which they were maintained with no further exposure for an additional 20 months. Histopathological examination revealed one adenocarcinoma and one adenomatous lesion of the lung in the low-exposure rats and one adenomatosis in the high-exposure group. Bronchial glandular hyperplasia was seen in five and six rats in the low- and high-dose groups, respectively; a malignant histiocytoma that emanated from the paranasal region was noted in the upper respiratory tract of one rat [group unspecified]. None of the five control rats developed these lesions, although both control and exposed animals exhibited some squamous metaplasia (Horie *et al.*, 1985). [The Working Group noted the small number of animals used and the short exposure period.]

Groups of 40 and 20 male Wistar rats, five weeks of age, were exposed by inhalation to 60 and 200 μg/m³ nickel as nickel monoxide aerosol (particle size, <0.3 μm) continuously for 18 months, followed by an observation period of one year under normal atmospheric conditions. At 24 months, 80% of animals in the treatment group had died, and at termination of the study (30 months) 62.5% of controls had died. No carcinogenic effect was observed (Glaser *et al.*, 1986). [The Working Group noted that the toxic effects, particularly alveolar proteinosis, were severe, that the survival of the animals was too short for carcinogenicity to be evaluated fully, and that nickel oxide aerosols were generated by atomization of aqueous nickel acetate solutions.]

Hamster: A group of 51 male Syrian golden hamsters, two months of age, was exposed by inhalation to a mean aerosol concentration of 53.2 mg/m³ nickel monoxide (mean particle diameter, 0.3 μm) for 7 h per day on five days per week for life. Another group of 51 males was exposed to nickel monoxide plus cigarette smoke. Two control groups of 51 animals were exposed to smoke and sham dust or to sham smoke and sham dust. Massive pneumoconiosis with lung consolidation developed in the nickel monoxide-exposed animals but did not affect their lifespan. Mean lifespan was 19.6 ± 1.6 months for animals exposed to smoke and nickel monoxide, 16.1 ± 1.1 for sham-exposed nickel oxide-treated animals and 19.6 ± 1.4 and 15.3 ± 1.3 months for the respective controls. No significant increase in the incidence of respiratory tumours or any evidence of cocarcinogenic interaction with cigarette smoke was noted for nickel monoxide. One osteosarcoma occurred in the nickel monoxide-treated group and one osteosarcoma and one rhabdomyosarcoma in the muscle of the thorax were seen in the group given nickel monoxide plus cigarette smoke (Wehner *et al.*, 1975, 1979).

(ii) *Intratracheal instillation*

Rat: Groups of female Wistar rats [numbers unspecified], 11 weeks of age, received ten weekly intratracheal instillations of 5 or 15 mg nickel as nickel monoxide (99.99% pure) in 0.3 ml saline to give total doses of 50 and 150 mg nickel, respectively. A control group of 40 rats received injections of saline only and were observed for 124 weeks. Lung tumour incidence in the two treated groups was 10/37 (27%) and 12/38 (31.6%), respectively; the tumours in the two groups consisted of four adenocarcinomas, two mixed tumours and 16 squamous-cell carcinomas. No lung tumour occurred in controls (Pott *et al.*, 1987).

Hamster: In an experiment designed to study the effects of particulates on the carcinogenesis of *N*-nitrosodiethylamine, groups of 25 male and 25 female hamsters [strain unspecified], five weeks old, received intratracheal instillations of 0.2 ml of a suspension of 2 g nickel monoxide (particle size, 0.5-1.0 μm) in 100 ml 0.5% w/v gelatin/saline once a week for 30 weeks. A group of 50 controls received injections

of carbon dust in the vehicle. Only three hamsters in each group survived beyond 48 weeks. One respiratory tract tumour [unspecified] was found in the 47 nickel monoxide-treated animals that were necropsied and four in controls. A high incidence of respiratory-tract tumours was observed in animals treated with *N*-nitrosodiethylamine alone (Farrell & Davis, 1974). [The Working Group noted the poor survival of treated and control animals.]

(iii) *Intrapleural administration*

Rat: A group of 32 male Wistar rats, three months of age, received a single intrapleural injection of 10 mg nickel monoxide in 0.4 ml saline suspension. A positive control group of 32 rats received a 10 mg injection of crocidolite, and a negative control group of 32 rats received saline alone. After 30 months, 31/32 rats in the nickel monoxide-treated group had developed injection-site tumours (mostly rhabdomyosarcomas). Median survival time was 224 days. Nine of 32 rats in the crocidolite-treated group had local tumours, but none of the saline controls developed local sarcomas (Skaug *et al.*, 1985).

(iv) *Intramuscular administration*

Mouse: Two groups of 50 Swiss and 52 C3H mice, equally divided by sex, two to three months of age, received single intramuscular injections of 5 mg nickel monoxide in penicillin G procaine into each thigh muscle and were observed for up to 476 days. Local sarcomas (mainly fibrosarcomas) occurred in 33 Swiss and 23 C3H mice. No control was reported (Gilman, 1962).

Rat: A group of 32 Wistar rats [sex unspecified], two to three months of age, received single intramuscular injections of 20 mg nickel monoxide powder into each thigh muscle and were observed for up to 595 days. Twenty-one rats developed a total of 26 tumours at the site of injection; 80% of the tumours were rhabdomyosarcomas, and the average latent period was 302 days. No control was reported (Gilman, 1962).

Groups of 20 Fischer rats [sex and age unspecified] received single intramuscular injections at two sites of either nickel hydroxide or nickel monoxide [dose unspecified] in aqueous penicillin G procaine. Local sarcomas developed in 15/20 (19 tumours at 40 sites) and 2/20 rats, respectively. Concurrent vehicle controls were not used. Seventeen of 20 animals given nickel subsulfide [dose unspecified] as positive controls developed local sarcomas. No tumour developed at the injection sites in two other groups of rats in the same experimental series injected intramuscularly with either nickel sulfate or nickel sulfide [presumed to be amorphous] (Gilman, 1966).

Ten male and ten female Wistar rats, weighing 150-170 g, received an intramuscular injection of 3 mg nickel trioxide powder. No control group was reported. No neoplasm developed at the injection site (Sosiński, 1975).

A group of 15 male Fischer 344 rats, two months of age, received a single intramuscular injection of nickel at 14 mg/rat as nickel monoxide (bunsenite, green-grey (Sunderman, 1984); 99.9% pure; particle diameter, <2 μm) in 0.3 ml of a 1:1 v/v glycerol:water vehicle into the right thigh and were observed for 104 weeks. Fourteen animals developed local sarcomas (mostly rhabdomyosarcomas) with a median tumour latency of 49 weeks and a median survival time of 58 weeks; metastases occurred in 4/14 rats. None of 40 control rats injected with vehicle alone developed tumours at the site of injection; 25/40 control rats were still alive at termination of the experiment (Sunderman & McCully, 1983).

Groups of 20 male Wistar rats, weighing 200-220 g, received a single intramuscular injection of 120 μmol [7.1 mg] nickel as one of three nickel hydroxide preparations — an air-dried gel, crystalline industrial nickel hydroxide and a freshly prepared colloidal nickel hydroxide — in 0.1 ml distilled water. A positive control group was treated with 120 μmol [7.1 mg] nickel as nickel subsulfide (see also p. 337) and a negative control group was treated with sodium sulfate. Seven rats treated with the colloidal preparation and one treated with the gel died from haematuria one to two weeks after the treatment. Six ulcerating, tumour-like growths developed between five and six months after treatment in the crystalline-treated group, but these regressed and were not included in tabulations. Local tumours occurred in 5/19 rats (four rhabdomyosarcomas, one fibrosarcoma) given the dried gel, 3/20 (all rhabdomyosarcomas) given the crystalline compound, 0/13 given the colloidal preparation, 16/20 positive controls and 0/20 negative controls (Kasprzak *et al.*, 1983). [See also pp. 360-361.]

In the study by Berry *et al.* (1984) described on p. 321, no tumour was induced by 20 mg nickel monoxide by either the intramuscular or subperiosteal route in groups of 20 rats.

In the study by Judde *et al.* (1987) described on p. 321, no tumour was induced by 20 mg nickel trioxide in ten rats.

(v) *Intraperitoneal administration*

Rat: A group of 50 female Wistar rats, 12 weeks of age, received two intraperitoneal injections of 500 mg nickel as nickel monoxide (99.99% pure); 46/47 of the animals developed abdominal tumours (sarcomas, mesotheliomas or carcinomas) with an average tumour latency of 31 months. Concurrent controls were not reported but, in other groups of saline controls, the incidence of abdominal tumours ranged from 0 to 6% (Pott *et al.*, 1987).

In a study described earlier (p. 322), single injections of 25 and 100 mg nickel as nickel monoxide induced local sarcomas and mesotheliomas in the peritoneal cavity in 12/34 and 15/36 female Wistar rats, respectively, after 30 months (Pott *et al.*,

1989, 1990). [The Working Group noted that the results at 30 months were available as an extended abstract only.]

(vi) *Intrarenal administration*

Rat: A group of 12 male Fischer 344 rats, two months of age, received an injection of nickel monoxide (green; 7 mg/rat nickel) in 0.1 or 0.2 ml saline into each pole of the right kidney and were observed for two years. No renal carcinoma was observed (Sunderman *et al.*, 1984b; see also p. 323).

(vii) *Intracerebral injection*

Rat: A group of ten male and ten female Wistar rats, weighing 150-170 g, received an intracerebral injection of 3 mg nickel trioxide powder into the cerebral cortex. No control group was reported. Cerebral sarcomas [gliomas] were observed in two rats that were killed at 14 and 21 months, respectively, and a meningioma was found in one rat that was killed at 21 months (Sosiński, 1975).

(*c*) *Nickel sulfides*

The experiments described below refer primarily to α-nickel subsulfide and to other crystalline forms of nickel sulfide, except where specifically stated that an amorphous form was tested.

(i) *Inhalation*

Rat: A group of 122 male and 104 female Fischer 344 rats [age unspecified] was exposed by inhalation to 0.97 mg/m^3 nickel subsulfide (particle diameter, < 1.5 μm) for 6 h per day on five days per week for 78 weeks. The remaining rats were observed for another 30 weeks, by which time survival was less than 5%. Survival of a group of 241 control rats exposed to filtered room air was 31% at 108 weeks. A significant increase in the incidence of benign and malignant lung tumours was observed compared to controls. Among treated rats, 14 malignant (ten adenocarcinomas, three squamous-cell carcinomas, one fibrosarcoma) and 15 benign lung tumour-bearing animals were identified; one adenocarcinoma and one adenoma developed among controls. The earliest tumour appeared at 76 weeks, and the average tumour latency was approximately two years. An elevated incidence of hyperplastic and metaplastic lung lesions was also noted among nickel subsulfide-treated rats (Ottolenghi *et al.*, 1974).

(ii) *Intratracheal instillation*

Mouse: Groups of 20 male B6C3F1 mice, eight weeks of age, received intratracheal instillations of 0.024, 0.056, 0.156, 0.412 or 1.1 mg/kg bw nickel subsulfide (particle size, < 2 μm) in saline once a week for four weeks and were observed for up to 27 months, at which time about 50% of the animals had died. Lung tumours

occurred in all groups; no significant difference from controls and no dose-response relationship was observed. No damage to the respiratory tract that was attributable to treatment was seen (Fisher *et al.*, 1986). [The Working Group noted the low doses used.]

Rat: Groups of 47, 45 and 40 female Wistar rats, 11 weeks of age, received intratracheal instillations of 0.063, 0.125 or 0.25 mg/animal nickel subsulfide in 0.3 ml saline (total doses, 0.94, 1.88 and 3.75 mg/animal) once a week for 15 weeks. At 120 weeks, 50% of the animals were still alive; the experiment was terminated at 132 weeks. The incidences of malignant lung tumours were 7/47, 13/45 and 12/40 in the low-, medium- and high-dose groups; 12 adenocarcinomas, 15 squamous-cell carcinomas and five mixed tumours occurred in the lungs of treated animals. No lung tumour occurred in 40 controls given 20 intratracheal injections of 0.3 ml saline (Pott *et al.*, 1987).

Hamster: In the study reported on p. 320 (Muhle *et al.*, 1990), no lung tumour was seen in 62 animals given 12 doses of 0.1 mg α-nickel subsulfide by intratracheal instillation. [The Working Group noted the low total dose given.]

(iii) *Intrapleural administration*

Rat: A group of 32 male Wistar rats, three months of age, received a single intrapleural injection of 10 mg nickel subsulfide in 0.4 ml saline. Average survival was 177 days. Local malignant tumours (mainly rhabdomyosarcomas) developed in 28/32 animals but in none of 32 saline-injected controls (Skaug *et al.*, 1985)

(iv) *Topical administration*

Hamster: Groups of male golden Syrian hamsters of the LVG/LAK strain, two to three months of age, were painted on the mucosa of the buccal pouches with 1 or 2 mg α-nickel subsulfide in 0.1 ml glycerol three times a week for 18 weeks (six to seven animals; total doses, 54 and 108 mg nickel subsulfide) or with 5 or 10 mg three times a week for 36 weeks (13-15 animals; total doses, 540 and 1080 mg nickel subsulfide), and were observed for more than 19 months. Two control groups received applications of glycerol. No tumour developed in the buccal pouch, oral cavity or intestinal tract in the treated or control groups. Squamous-cell carcinomas of the buccal pouch developed in all four hamsters that received applications of 1 mg dimethylbenz[*a*]anthracene in glycerol three times a week for 18 weeks (Sunderman, 1983b).

(v) *Intramuscular administration*

Mouse: Groups of 45 Swiss and 18 C3H mice, approximately equally divided by sex, two to three months of age, received single intramuscular injections of 5 mg nickel subsulfide into both or only one thigh muscle. Local tumours (mainly sarco-

mas) developed in 27 and nine mice, respectively. No control was reported (Gilman, 1962).

Three groups of ten female and one group of ten male NMRI mice, six weeks of age, received an injection of 10 mg labelled nickel subsulfide into the left thigh muscle, or of 5 mg into the interscapular subcutaneous tissue, in 0.1 ml olive oil:streptocillin (3:1). Two mice from each group were killed two months after injection for whole-body autoradiography; no tumour was seen at this stage. The remaining animals were autopsied at 14 months, when local sarcomas were seen in 7/8 and 4/8 females that received subcutaneous injections and in 4/8 males and 4/8 females that received intramuscular injections. Metastases to the lung, liver and regional lymph nodes occurred in approximately half of the 19 tumour-bearing mice. No control group was used (Oskarsson *et al.*, 1979).

Groups of four male and six female DBA/2 and five male and five female C57Bl6 mice, two to three months of age, received a single intramuscular injection of 2.5 mg α-nickel subsulfide in 0.1-0.5 ml penicillin G procaine solution into one thigh muscle. Local sarcomas developed in six DBA/2 ($p < 0.01$) and in five C57Bl6 ($p < 0.05$) mice, with median latent periods of 13 and 14 months, respectively. None of nine control mice of each strain injected with penicillin G alone developed a sarcoma (Sunderman, 1983b).

Rat: A group of 32 male and female Wistar rats, two to three months of age, received a single intramuscular injection of 20 mg nickel subsulfide into one or both thigh muscles. After an average of 21 weeks, 25/28 rats had developed 36 local tumours. Vehicle controls were not available, but two further groups of 30 rats each injected with ferrous sulfide did not develop tumours at the site of injection after 627 days (Gilman, 1962).

Groups of ten male and ten female Fischer rats, five months of age, were administered nickel subsulfide either by an intramuscular injection of 10 mg powder (particle size, 2-4 μm), by implantation of an intact 11-mm disc (500 mg), by implantation of 3-5-mm disc fragments or by implantation of 10 mg powder in a 0.45-μm porosity millipore diffusion chamber. Local tumours (mostly rhabdomysarcomas) developed in 71-95% of rats, which demonstrated diffusion of soluble nickel from the chambers. The mean tumour latency for the last group was 305 days, almost twice that for the other three groups. Among 19 controls given 38 implants of empty diffusion chambers, one tumour developed after 460 days. The authors considered that the experiment demonstrated that the induction of neoplasms by nickel subsulfide is a chemical rather than a physical (foreign-body) reaction and that phagocytosis is not essential for nickel tumorigenesis (Gilman & Herchen, 1963).

Groups of 15 Fischer rats received implants of nickel subsulfide discs (250 mg) or 8 × 1-mm discs of ferric oxide (control) in opposite sides of the gluteal

musculature. The nickel subsulfide discs were removed in a geometric sequence at two, four, eight... up to 256 days after implantation, and average tumour incidence after 256 days was 66%. The critical exposure (tissue contact) period necessary for nickel subsulfide to induce malignant transformation was 32-64 days (Herchen & Gilman, 1964).

Groups of 15 male and 15 female hooded and 15 male and 12 female NIH (Bethesda) black rats, two to three months of age, received injections of 10 mg nickel subsulfide in penicillin G procaine into each gastrocnemius muscle. NIH Black rats were less susceptible to local tumour induction (14/23 rats) than hooded rats (28/28). Massive phagocytic invasion of the nickel injection site occurred in the NIH black rats (Daniel, 1966).

Groups of 20 male and 20 female Fischer 344 rats, five weeks of age, received a single subcutaneous injection of 10 or 3.3 mg nickel subsulfide in 0.25 ml saline. Two further groups received single intramuscular injections of 10 or 3.3 mg nickel subsulfide. A group of 60 male and 60 female control rats received injections of 0.25 ml saline twice a week for 52 weeks, and a further control group received no treatment. At 18 months, the groups injected subcutaneously with nickel subsulfide had tumour incidences of 90 and 95%, and the groups injected intramuscularly had tumour incidences of 85% and 97%. Most tumours in both groups were rhabdomyosarcomas. No local tumour occurred in controls (Mason, 1972).

Groups of ten male Fischer 344 rats, three months of age, received intramuscular injections of amorphous nickel sulfide and α-nickel subsulfide in 0.5 ml penicillin G procaine suspension at two comparable dose levels (about 5 and 20 mg/rat), to provide 60 and 240 μg Ni per rat. A further group received injections of nickel ferrosulfide matte (85 and 340 μg atom of nickel per rat). Sarcomas at the injection site developed in 8/10 and 9/9 of the low- and high-dose nickel subsulfide-treated groups and in 1/10 and 8/10 of the low- and high-dose nickel ferrosulfide matte-treated groups, respectively. No local sarcoma developed in the groups given nickel sulfide, among control rats given penicillin G procaine suspension alone or in two control groups treated with metallic iron powder (Sunderman & Maenza, 1976).

Groups of 63 male and female inbred Fischer and 20 male and female hooded rats, ten to 14 weeks old, received an intramuscular injection of 10 mg nickel subsulfide in penicillin G procaine. Tumour-bearing rats were autopsied 30 days after detection of the tumour. Tumours occurred in 59/63 Fischer and 11/20 hooded rats; 81.9% of tumours in hooded rats metastasized, compared to 25.4% in Fischer rats. Metastatic lesions were observed in the heart, pleura, liver and adrenal glands, as well as in lungs and lymph nodes of nine hooded rats. Of the primary tumours, 67% were rhabdomyosarcomas (Yamashiro *et al.*, 1980).

Groups of 30 male Fischer 344 rats, approximately two months of age, received a single intramuscular injection of 0.6, 1.2, 2.5 or 5 mg nickel subsulfide. Local sarcomas were recorded in 7/30, 23/30, 28/30 and 29/30 of the animals, respectively [$p < 0.01$], indicating a dose-related increase in incidence. No such tumour developed in 60 untreated controls (Sunderman *et al.*, 1976). In an extension of this study, a total of 383 animals received injections of 0.63-20 mg α-nickel subsulfide. Sarcoma incidence at 62 weeks after treatment ranged from 24% at the lowest dose level to 100% at the highest dose level. Of the 336 sarcomas induced, 161 were rhabdomyosarcomas, 91 undifferentiated sarcomas, 72 fibrosarcomas, nine liposarcomas, two neurofibrosarcomas and one a haemangiosarcoma. Metastasis was seen in 137 of the 336 tumour-bearing animals (Sunderman, 1981).

In a study on the relationship between physical and chemical properties and carcinogenic activities of 18 nickel compounds at a standard 14-mg intramuscular dose of nickel under comparable experimental conditions in male Fischer 344 rats (see p. 321), five nickel sulfides were among the compounds tested. Three of these (α-nickel subsulfide, crystalline β-nickel sulfide and nickel ferrosulfide matte) induced local sarcomas in 100% of animals (9/9, 14/14 and 15/15). Metastases developed in 56, 71 and 67%, respectively, of the tumour-bearing rats. Nickel disulfide induced local tumours in 86% (12/14) animals and amorphous nickel sulfide in 12% (3/25). Median latent periods were 30 weeks for nickel subsulfide, 40 weeks for crystalline nickel sulfide, 36 weeks for nickel disulfide, 41 weeks for amporphous nickel sulfide, but only 16 weeks for nickel ferrosulfide. Median survival times were 39, 48, 47, 71 and 32 weeks, respectively (Sunderman, 1984).

In the study by Berry *et al.* (1984) described on p. 321, tumours developed in 10/20 rats given 5 mg nickel subsulfide intramuscularly, in 0/20 treated subperiosteally and in 10/20 given intrafemoral injections.

In the study by Judde *et al.* (1987) described on p. 321, a single intramuscular injection of 5 mg nickel subsulfide induced tumours in 2/100 rats.

[The Working Group was aware of several other studies in which nickel subsulfide was used as a positive control or as a model for the induction of rhabdomyosarcomas.]

Hamster: Groups of 15 or 17 male Syrian hamsters, two to three months of age, received a single intramuscular injection of 5 or 10 mg nickel subsulfide in 0.02-0.5 ml sterile saline. Of the 15 animals receiving the 5-mg dose, four developed local sarcomas, with a median latent period of ten months. At the 10-mg dose, 12/17 hamsters had local tumours, with a mean latency of 11 months [$p < 0.01$, trend test]. No tumour occurred among 14 controls injected with saline alone (Sunderman, 1983a).

Rabbit: Six-month-old white rabbits [sex and number unspecified] received intramuscular implants of agar-agar blocks containing approximately 80 mg nickel subsulfide powder. Sixteen rabbits with local tumours (rhabdomyosarcomas) were examined. Tumours were first observed about four to six months after implantation as small growths, which usually ceased active progression for up to 80 weeks then grew rapidly over the next four or five weeks (Hildebrand & Biserte, 1979a,b). [The Working Group noted the limited reporting of the study.]

Four male New Zealand albino rabbits, two months old, received bilateral intramuscular injections of 25 mg α-nickel subsulfide (50 mg/rabbit) in 0.1-0.5 ml penicillin G procaine suspension. All animals died between 16 and 72 months. No local tumour was found on autopsy (Sunderman, 1983a). [The Working Group noted the short observation period.]

(vi) *Intraperitoneal administration*

Rat: Of a group of 37 Fischer rats [sex and age unspecified] that received a single intraperitoneal injection of nickel subsulfide [dose unspecified], nine developed tumours, eight of which were rhabdomyosarcomas and one a mesothelioma (Gilman, 1966). [The Working Group noted the limited reporting of the study.]

A group of 50 female Wistar rats, 12 weeks of age, received a single intraperitoneal injection of 25 mg nickel subsulfide. Abdominal tumours (sarcomas, mesotheliomas and carcinomas) occurred in 27/42 animals, with an average latent period of eight months (Pott *et al.*, 1987).

In a study described above (p. 322), three doses of nickel subsulfide were injected into the peritoneal cavities of groups of female Wistar rats. Local tumours were observed at 30 months in 20/36 animals that received 6 mg (as Ni) as a single injection, in 23/35 receiving 12 mg (as Ni) as two 6-mg injections and in 25/34 given 25 mg (as Ni) as 25 1-mg injections. The tumours were mesotheliomas or sarcomas of the abdominal cavity (Pott *et al.*, 1989, 1990). [The Working Group noted that the results at 30 months were available as an extended abstract only.]

(vii) *Intrarenal administration*

Rat: Groups of 16 and 24 female Sprague-Dawley rats, weighing 120-140 g, received a single injection of 5 mg nickel subsulfide in 0.05 ml glycerine or 0.5 ml saline into each pole of the right kidney. Renal-cell carcinomas occurred in 7/16 and 11/24 animals compared with 0/16 in animals given 0.5 ml glycerine (Jasmin & Riopelle, 1976).

In a second experiment (Jasmin & Riopelle, 1976), the activity of other nickel compounds and divalent metals was investigated under identical experimental conditions using glycerine as the vehicle; all rats were autopsied after 12 months' exposure. In one group of 18 rats, nickel sulfide [probably amorphous] exhibited no

renal tumorigenic activity. [The Working Group noted that it was not stated whether crystalline or amorphous nickel sulfide was used.]

Groups of male and female Wistar Lewis, NIH black, Fischer 344 and Long-Evans rats, eight weeks of age, received an intrarenal injection of 5 mg α-nickel subsulfide. The incidence of malignant renal tumours 100 weeks after exposure was 7/11 in Wistar Lewis, 6/12 in NIH black, 9/32 in Fischer and 0/12 in Long-Evans rats. Groups of 11-24 male Fischer rats were given an intrarenal injection of 0.6, 1.2, 2.5, 5 or 10 mg nickel subsulfide; no tumour was seen with 0.6, 1.2 or 2.5 mg, but responses of 5/18 and 18/24 were obtained with 5 mg and 10 mg, showing a dose-response effect. All tumours were malignant, but the authors could not establish whether the tumours were of epithelial or mesenchymal origin; 70% had distant metastases (Sunderman *et al.*, 1979a).

Groups of male Fischer 344 rats [initial number unspecified], approximately eight weeks old, received an intrarenal injection of 7 mg nickel as one of several sulfides in 0.1 or 0.2 ml saline or in glycerol:distilled water (1:1, v/v) in each pole of the right kidney and were observed for two years after treatment. The incidence of renal cancer was significantly elevated in treated groups: nickel disulfide, 2/10 (fibrosarcomas); crystalline β-nickel sulfide, 8/14 (three fibrosarcomas, three other sarcomas, one renal-cell carcinoma, one carcinosarcoma); and α-nickel subsulfide, 4/15 (mesangial-cell sarcomas). Renal cancers occurred in 1/12 (sarcoma) rats treated with nickel ferrosulfide and in 0/15 rats treated with amorphous nickel sulfide. No local tumour developed in vehicle controls (Sunderman *et al.*, 1984b).

(viii) *Intratesticular administration*

Rat: A group of 19 male Fischer 344 rats, eight weeks of age, received an injection of 10 mg α-nickel subsulfide in 0.3 ml saline into the centre of the right testis and were observed for 20 months, at which time all the animals had died. A control group of 18 rats received an injection of 0.3 ml saline only, and a further two groups of four rats each received injections of either 10 mg metallic iron powder in saline or 2 mg zinc[II] as zinc chloride in distilled water. Of the nickel subsulfide-treated rats, 16/19 developed sarcomas in the treated testis, ten of which were fibrosarcomas, three malignant fibrous histiocytomas and three rhabdomyosarcomas. Four of the rats had distant metastases. No tumour occurred in the other groups (Damjanov *et al.*, 1978).

(ix) *Intraocular administration*

Rat: A group of 14 male and one female Fischer 344 rats, four weeks of age, received an injection of 0.5 mg α-nickel subsulfide in 20 μl saline into the vitreous cavity of the right eye under anaesthetic. Eleven male controls were similarly injected with saline alone. The experiment was terminated at 40-42 weeks after

treatment, when 11 control and one surviving treated rats were killed. Between 26 and 36 weeks after injection, 14/15 rats developed ocular tumours. Five of the tumorous eyes contained multiple neoplasms, and 22 distinct ocular tumours were identified as 11 melanomas, four retinoblastomas, three gliomas, one phakocarcinoma [lens capsular tumour] and three unclassified malignant tumours. No tumour developed in either the controls or in the uninjected, left eyes of treated rats. It was postulated that the very high incidence (93%) and short latent periods may have been due in part to the relative isolation of the vitreous bodies from the systemic circulation (blood-retina barrier), which would result in a high concentration of nickel[II]. The authors also pointed out that nickel particles within the vitreous body were relatively sequestered from phagocytosis. The visibility of developing tumours within the chamber permits their very early recognition (Albert *et al.*, 1980; Sunderman, 1983b).

Salamander: A group of eight lentectomized Japanese common newts received a single injection of 40-100 μg nickel subsulfide into the vitreous chamber of the eye under anaesthetic. Seven newts developed ocular melanoma-like tumours within nine months, while no tumour occurred in six controls injected with 2-3 μl sterile 0.6% saline or eye-dropper oil after lens extirpation. The lens regenerated in each of the control eyes. The site of tumour origin could not be determined, although it was suggested to be the iris, which showed numerous aberrant proliferating cells at three months (Okamoto, 1987).

(x) *Transplacental administration*

Rat: A group of eight pregnant female Fischer 344 rats, 120-150 days of age, received an intramuscular injection of 20 mg α-nickel subsulfide in 0.2 ml procaine penicillin G suspension on day 6 of gestation, allowing for gradual dissolution of the nickel subsulfide throughout the remainder of the pregnancy. A group of controls received an injection of vehicle only. No difference in the incidence of benign or malignant tumours was seen between the 50 pups born to treated dams and 53 control pups observed for 26 months (Sunderman *et al.*, 1981). [The Working Group noted that only one dose was used, which was not toxic to the fetuses.]

(xi) *Implantation into subcutaneously implanted tracheal grafts*

Rat: Groups of 30 and 32 female Fischer 344 rats, ten weeks of age, received five gelatin pellets containing 1 or 3 mg nickel subsulfide in heterotopic tracheal transplants inserted under the dorsal skin. At the lower dose level, tumours developed in 9/60 tracheas (six carcinomas and three sarcomas); at the higher dose level, tumours developed in 45/64 tracheas (one carcinoma and 44 sarcomas). No tumour developed in 20 control transplanted tracheas. The high dose resulted in necrosis of the epithelium and thus favoured the development of sarcomas (Yarita & Nettesheim, 1978).

(xii) *Intramuscular, subcutaneous or intra-articular injection or injection into retroperitoneal fat*

Rat: In a study designed to determine the types of sarcoma that develop from various mesenchymal tissue components, groups of 20 male Fischer 344 rats, seven to eight weeks of age, received injections of 5 mg nickel subsulfide either intramuscularly, subcutaneously, into the intra-articular space or into retroperitoneal fat. Control groups of ten rats each were injected with 0.5 ml aqueous procaine penicillin G vehicle. The incidences and types of sarcoma that developed in the experimental groups were: intramuscular, 19/20 (all rhabdomyosarcomas); subcutaneous, 18/19 (ten malignant fibrous histiocytomas, five rhabdomyosarcomas, three fibrosarcomas or unclassified); intra-articular, 16/19 (eight rhabdomyosarcomas, three malignant fibrous histiocytomas, five fibrosarcomas or unclassified); and retroperitoneal fat, 9/20 (five malignant fibrous histiocytomas, three rhabdomyosarcomas, one fibrosarcoma or unclassified). Controls did not develop tumours (Shibata *et al.*, 1989).

(xiii) *Administration with known carcinogens*

Rat: Groups of 30 male Fischer rats, eight to nine weeks of age, received intramuscular injections in both thighs of either 10 mg nickel subsulfide, 10 mg benzo[*a*]pyrene or 20 mg nickel subsulfide plus 10 mg benzo[*a*]pyrene in penicillin G procaine suspension, or vehicle alone. All treated rats developed sarcomas; rhabdomyosarcomas occurred in 24/30 given 10 mg nickel subsulfide, 4/30 given benzo[*a*]pyrene and 28/30 given 20 mg nickel subsulfide plus benzo[*a*]pyrene. No sarcoma occurred in controls (Maenza *et al.*, 1971).

Groups of 13, 13 and 12 male Wistar rats, weighing approximately 200 g, received single intratracheal injections of 5 mg nickel subsulfide, 2 mg benzo[*a*]pyrene or 5 mg nickel subsulfide plus 2 mg benzo[*a*]pyrene and were observed for 15 months. One rat from each group developed a tumour, consisting of one hepatoma, one retroperitoneal tumour and one squamous-cell carcinoma of the lung, respectively. Significant differences were seen in the incidence of preneoplastic lesions (peribronchial adenomatoid proliferation and bronchial squamous metaplasia), the occurrence decreasing in the order: nickel subsulfide plus benzo[*a*]pyrene > benzo[*a*]pyrene > nickel subsulfide (Kasprzak *et al.*, 1973).

(*d*) *Nickel salts*

(i) *Intramuscular administration*

Rat: A group of 32 male and female Wistar rats, two to three months of age, received an injection of 5 mg nickel sulfate hexahydrate in one or both thigh muscles (54 injected sites). Thirteen rats survived until the end of the experiment at 603 days.

No local tumour was found at the site of injection. No vehicle control was used (Gilman, 1962).

In a study reported as an abstract, sheep fat pellets, each containing 7 mg of either nickel sulfate, nickel chloride, nickel acetate, anhydrous nickel acetate, nickel carbonate or nickel ammonium sulfate, were given as three intramuscular implants [interval unspecified] into groups of 35 Bethesda black [NIH black] rats. Animals were observed for 18 months. Six tumours developed in the nickel carbonate group; single tumours developed in the nickel acetate and nickel sulfate groups. No tumour developed in any of the other groups or in 35 controls (Payne, 1964).

In a study comparing the in-vitro solubility and carcinogenicity of several nickel compounds, nickel fluoride and nickel sulfate were suspended in penicillin G procaine and injected intramuscularly [dose unspecified] into groups of 20 Fischer rats [sex and age unspecified]. The incidence of local sarcomas was 3/18 (17%; 3/36 sites) with nickel fluoride and 0/20 with nickel sulfate. Seventeen of 20 (85%) rats given nickel subsulfide as a positive control developed local sarcomas. No tumour developed in 20 rats injected with nickel sulfide [presumed to be amorphous] (Gilman, 1966). [The Working Group noted that no concurrent vehicle control was used and that the length of observation was not specified.]

A group of 20 male Wistar rats, weighing 200-220 g, received 15 intramuscular injections of 20 µl of a 0.2 M solution of nickel sulfate (4.4 µmol [0.26 mg]/injection of nickel; total dose, 66 µmol [4 mg]/rat nickel) every other day during one month. Further groups of 20 male rats received injections of nickel subsulfide (total dose, 40 µmol [7.1 mg nickel]; positive control) or sodium sulfate (15 injections of 20 µl of a 0.2 M solution; negative control). Nickel subsulfide induced local tumours in 16/20 rats; no tumour developed in nickel sulfate- or sodium sulfate-treated rats (Kasprzak *et al.*, 1983).

One local sarcoma was found in 16 male Fischer 344 rats, two to three months old, given an intramuscular injection of nickel chromate into the right thigh as 14 mg/rat nickel. Ten rats survived two years (Sunderman, 1984).

(ii) *Intraperitoneal administration*

Mouse: In a screening assay for lung adenomas in strain A mice, groups of ten male and ten female Strong strain A mice, six to eight weeks old, received intraperitoneal injections of nickel acetate in 0.85% physiological saline (total doses, 72, 180 and 360 mg/kg bw) three times a week for 24 weeks and were observed for 30 weeks, at which time all survivors were autopsied. Further groups of mice received a single intraperitoneal injection of 20 mg urethane (positive control), 24 injections of saline only or remained untreated. The incidences of lung tumours were: saline control, 37% (0.42 tumours/animal); untreated control, 31% (0.28 tumours/animal); positive control, 100% (21.6 tumours/animal); 72 mg nickel acetate, 44% (0.67 tumours/

animal); 180 mg nickel acetate, 50% (0.71 tumours/animal); and 360 mg nickel acetate, 63% (1.26 tumours/animal). The difference in response between the group given 360 mg nickel acetate and the negative control group was significant ($p < 0.01$). Five adenocarcinomas of the lung were observed in the nickel-treated mice compared to none in controls (Stoner *et al.*, 1976).

In the same type of screening assay, 30 male and female Strong strain A mice, six to eight weeks of age, received intraperitoneal injections of 10.7 mg/kg bw nickel acetate tetrahydrate (maximal tolerated dose; 0.04 mmol [2.4 mg]/kg bw nickel) three times a week for 24 weeks. A control group received injections of 0.9% saline under the same schedule. Animals were autopsied 30 weeks after the first injection. Of the nickel-treated group, 24/30 animals survived to 30 weeks and had an average of 1.50 lung adenomas/animal, whereas 25/30 controls had an average of 0.32 lung adenoma/animal ($p < 0.05$) (Poirier *et al.*, 1984).

Rat: In a study described earlier (p. 322), groups of female Wistar rats were given repeated intraperitoneal injections of 1 mg of each of four soluble nickel salts. The dose schedule and tumour responses at 30 months are shown in Table 23. The tumours were either mesotheliomas or sarcomas (tumours of the uterus were not included) (Pott *et al.*, 1989, 1990). [The Working Group noted that administration of nickel sulfate and nickel chloride by intramuscular injection has not been shown to induce tumours in rats. They suggest that in this instance the repeated small intraperitoneal doses permitted repeated exposure of potential target cells. Repeated intramuscular injections would result in nickel coming into contact with different cells at each injection. The Group also noted that the results at 30 months were reported only as an extended abstract.]

(iii) *Administration with known carcinogens*

Rat: Groups of 12 rats [strain, sex and age unspecified] received a single subcutaneous injection of 9 mg/ml dinitrosopiperazine in aqueous Tween 80. The following day, one group received topical insertion into the nasopharynx of 0.02 ml of a 0.5% solution of nickel sulfate in 4% aqueous gelatin once a week for seven weeks. A further group was held for six days and then administered 1 ml of aqueous 1% nickel sulfate solution in the drinking-water for six weeks. Additional groups of 12 rats received treatment with dinitrosopiperazine, nickel sulfate solution or nickel sulfate in gelatin only. Survival at 371 days was lower in the group treated with dinitropiperazine plus nickel sulfate solution in the drinking-water than in the group given the nitrosamine or the nickel sulfate solution alone. Two nasopharyngeal tumours (one squamous-cell carcinoma, one fibrosarcoma) occurred in the group treated with dinitropiperazine plus nickel sulfate in drinking-water and two (one papilloma, one early carcinoma) in the group treated with dinitropiperazine plus

Table 23. Tumour responses of rats to intraperitoneal injection of soluble nickel salts[a]

Compound	Total dose (mg, as Ni)	Schedule	Incidence of abdominal tumours
Nickel chloride.$6H_2O$	50	50 × 1 mg	4/32 [$p < 0.05$]
Nickel sulfate.$7H_2O$	50	50 × 1 mg	6/30 [$p < 0.05$]
Nickel acetate.$4H_2O$	25	25 × 1 mg	3/35
	50	50 × 1 mg	5/31 [$p < 0.05$ for trend]
Nickel carbonate	25	25 × 1 mg	1/35
Nickel hydroxide.$2H_2O$	50	50 × 1 mg	3/33
Saline		3 × 1 ml	1/33
		50 × 1 ml	0/34

[a]From Pott *et al.* (1989, 1990)

insertion of nickel sulfate in gelatin. No tumour occurred in the other groups. The authors concluded that 'probably nickel has a promoting action in the induction of nasopharyngeal carcinoma in rats following dinitrosopiperazine initiation' (Ou *et al.*, 1980). [The Working Group noted the small number of animals used and the poor survival.]

As reported in an abstract, in an extension of the study by Ou *et al.* (1980), five of 22 rats given an initiating injection of dinitrosopiperazine developed carcinomas following oral administration of nickel sulfate in gelatin. Two of the carcinomas were of the nasopharynx, two of the nasal cavity and one of the hard palate. No tumour developed in rats [numbers unspecified] treated with dinitrosopiperazine plus aqueous nickel sulfate, with nickel sulfate in gelatin alone or with dinitrosopiperazine alone (Liu *et al.*, 1983). [The Working Group noted the small number of animals used and the poor survival.]

As reported in an abstract, a group of 13 female rats [strain and age unspecified] received a single subcutaneous injection of 9 mg dinitrosopiperazine on day 18 of gestation. Pups of treated dams were fed 0.05 ml of 0.05% nickel sulfate beginning at four weeks of age every day for one month. The dose of nickel sulfate was increased by 0.1 ml per month for a further five months, by which time 5/21 pups had developed carcinomas of the nasal cavity. In a group of untreated pups of treated dams, 3/11 rats developed tumours (one nasopharyngeal squamous-cell carcinoma, one neurofibrosarcoma of the peritoneal cavity and one granulosa-thecal-cell carcinoma of the ovary). Groups given nickel sulfate and untreated control groups of seven pups each did not develop tumours. None of the pregnant rats that had been injected with dinitrosopiperazine alone developed tumours (Ou *et al.*, 1983).

Groups of 15 male Fischer 344 rats, seven weeks old, were administered 500 mg/l *N*-nitrosoethylhydroxyethylamine (NEHEA) in the drinking-water for two weeks. Thereafter, rats received drinking-water alone or drinking-water containing 600 mg/l nickel chloride hexahydrate for 25 weeks, when the study was terminated. The incidence of renal-cell tumours in the group receiving NEHEA and nickel chloride (8/15) was significantly higher ($p < 0.05$) than that in controls given NEHEA alone (2/15) or nickel chloride alone (0/15) (Kurokawa *et al.*, 1985). Nickel chloride did not show promoting activity in livers of Fischer 344 rats after initiation with *N*-nitrosodiethylamine, in gastric tissue of Wistar rats after initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, in the pancreas of Syrian golden hamsters following initiation with *N*-nitrosobis(2-oxypropyl)amine or in skin of SENCAR mice initiated with 7,12-dimethylbenz[*a*]anthracene. The authors concluded that nickel chloride is a promoter in renal carcinogenesis in rats (Hayashi *et al.*, 1984; Kurokawa *et al.*, 1985).

(*e*) *Other nickel compounds*

(i) *Inhalation*

Rat: Groups of 64 or 32 male Wistar rats, weighing 200-250 g, were exposed by inhalation for 30 min to 30 or 60 mg/m^3 nickel carbonyl vapourized from a solution in 50:50 ethanol:diethyl ether, respectively, three times a week for 52 weeks. Another group of 80 rats was exposed once to 250 mg/m^3 nickel carbonyl. All treated animals had died by 30 months. One lung carcinoma appeared in each of the first two groups, and two pulmonary carcinomas developed in the last group. No pulmonary tumour occurred among 41 vehicle-treated control rats (Sunderman *et al.*, 1957, 1959). A further group of 285 rats was exposed for 30 min to 600 mg/m^3 nickel carbonyl; 214 died from acute toxicity. One lung adenocarcinoma was observed in the remaining 71 animals. Similar exposure to nickel carbonyl followed by intraperitoneal injection of sodium diethyl dithiocarbamate, an antidote, resulted in survival of all 60 treated rats and the development of a single anaplastic lung carcinoma. Minimal time to observation of lung tumours in these groups was in excess of 24 months. No lung carcinoma was observed in a group of 32 controls (Sunderman & Donnelly, 1965).

A group of five non-inbred rats [sex and age unspecified] was exposed by inhalation to 70 mg/m^3 nickel refinery dust (containing 11.3% metallic nickel, 58.3% nickel sulfide [identity unspecified], 1.7% nickel monoxide and 0.2% water-soluble nickel [composition of sample unclear]) for 5 h per day on five days per week for six months. Seventeen months after the start of treatment, one of five rats developed a squamous-cell carcinoma of the lung. No tumour developed among 47 untreated controls (Saknyn & Blokhin, 1978). [The Working Group noted the small number of animals used.]

Hamster: Groups of 102 male Syrian golden outbred LAK:LVG hamsters, two months old, were exposed by inhalation to concentrations of 17 or 70 mg/m^3 nickel-enriched fly ash from the addition of nickel acetate to pulverized coal before combustion (nickel content, 6%) for 6 h per day on five days per week for 20 months. Further groups were exposed to 70 mg/m^3 fly ash containing 0.3% nickel, or were sham-exposed. Five animals from each group were autopsied at four-month intervals up to 16 months, and all survivors were sacrificed at 20 months. No significant difference in mortality rate or body weight was observed between the groups. There were 14, 16, 16 and seven benign and malignant tumours in the sham-exposed, fly ash, low-dose and high-dose nickel-enriched fly ash groups, respectively. The only two malignant pulmonary neoplasms (one adenocarcinoma, one mesothelioma) occurred in the group receiving fly ash enriched with the high dose of nickel (Wehner *et al.*, 1981, 1984).

(ii) *Intratracheal instillation*

Rat: A group of 26 white non-inbred rats [sex and age unspecified] received a single intratracheal instillation of 20-40 mg aerosol dust (64.7% nickel monoxide (black), 0.13% nickel sulfide, 0.18% metallic nickel) in 0.6 ml saline. One squamous-cell carcinoma of the lung had developed by 17 months. No tumour developed among a group of 47 controls (Saknyn & Blokhin, 1978). [The Working Group noted that it was not stated whether the controls were untreated or received the vehicle alone.]

(iii) *Intramuscular administration*

Mouse: A group of 40 female Swiss mice, two to three months of age, received an intramuscular injection in each thigh of 10 mg of a nickel refinery dust (57% nickel subsulfide, 20% nickel sulfate hexahydrate, 6.3% nickel monoxide) suspended in penicillin G procaine. Of the 36 mice that survived more than 90 days, 20 developed a total of 23 local sarcomas, with an average latent period of 46 weeks. No tumour occurred among 48 control mice injected with the vehicle alone (Gilman & Ruckerbauer, 1962).

Rat: A group of 35 male and female hooded rats, two to three months of age, received an intramuscular injection in each thigh of 20 mg of a nickel refinery dust (57% nickel subsulfide, 20% nickel sulfate hexahydrate, 6.3% nickel oxide) suspended in penicillin G procaine. Of the 27 rats that survived more than 90 days, 19 developed local sarcomas. Another group of 31 male and female rats received injections of the same refinery dust after repeated washing in distilled water; 20/28 of the rats that survived more than 90 days developed local tumours at one or other of the injection sites. No tumour occurred among 30 control rats injected with the vehicle alone (Gilman & Ruckerbauer, 1962).

Groups of 25 male and 25 female Fischer 344 rats [age unspecified] received 12 intramuscular injections of 12 or 25 mg nickelocene in trioctanoin. Tumour incidences were 18/50 and 21/50, respectively. No local tumour occurred in a group of 25 male and 25 female controls (Furst & Schlauder, 1971).

Groups of 15-30 male Fischer 344 rats, approximately eight weeks old, received a single intramuscular injection of 14 mg nickel as one of four nickel arsenides, nickel antimonide, nickel telluride, nickel sinter matte (Ni_4FeS_4; positive control), nickel titanate or ferronickel alloy ($NiFe_{1-6}$; negative controls) in 0.3 ml glycerol:water (1:1; v/v) into the exterior thigh. The compounds were >99.9% pure and were ground down to a median particle size of <2 μm. Rats that died within two months of the injection were excluded from the experiment; remaining animals were observed for two years. Median survival ranged from 32 weeks (positive controls) to over 100 weeks (negative controls). The incidences of local tumours in the groups were: nickel sinter matte, 15/15; nickel sulfarsenide, 14/16; nickel arsenide hexagonal, 17/20; nickel antimonide, 17/29; nickel telluride, 14/26; and nickel arsenide tetragonal, 8/16. No tumour was observed in the groups treated with nickel arsenide, ferronickel alloy or nickel titanate nor in a vehicle control group. Median latency for tumour induction ranged from 16 weeks (positive controls) to 33 weeks (nickel arsenide tetragonal-treated group). The incidence of tumours induced by the test compounds was significantly greater than that in the vehicle control group ($p < 0.001$); 67% of all the sarcomas were rhabdomyosarcomas, 11% fibrosarcomas, 15% osteosarcomas and 5% undifferentiated sarcomas. Metastases occurred in 57% of tumour-bearing rats (Sunderman & McCully, 1983).

In a continuation of these tests, nickel selenide, nickel subselenide and nickel monoxide (positive control; see p. 327) were tested using the same experimental techniques. Nickel selenide and nickel subselenide induced significant increases in the incidence of local tumours (8/16 and 21/23, respectively; $p < 0.001$); the positive control group had 14/15 tumours. Metastases occurred in 38 and 86%, respectively, of tumour-bearing rats in the selenium-treated groups and in 29% of positive controls. Approximately 50% of the tumours were rhabdomyosarcomas (Sunderman, 1984).

Hamster: Groups of 25 male and 25 female hamsters, three to four weeks old, received eight monthly injections of 5 mg nickelocene in 0.2 ml trioctanoin into the right thigh. No tumour was induced. A group of survivors from another test [age unspecified] received a single intramuscular injection of 25 mg nickelocene in trioctanoin; fibrosarcomas occurred in 1/13 females and 3/16 males. No tumour occurred in 25 male or 25 female vehicle controls (Furst & Schlauder, 1971).

(iv) *Intraperitoneal administration*

Rat: Groups of 16 and 23 non-inbred albino rats [sex and age unspecified] received a single intraperitoneal injection of 90-150 mg of one of two refinery dusts: the first contained 11.3% metallic nickel, 58.3% nickel sulfide, 1.7% nickel monoxide and 0.2% water-soluble nickel; the second contained 2.9% metallic nickel, 26.8% nickel sulfide, 6.8% nickel monoxide and 0.07% water-soluble nickel. Each was given in 1.5 ml physiological saline. Three local sarcomas developed within six to 15 months in animal treated with the first dust, and three local sarcomas developed within nine to 11 months in animals treated with the second dust. No tumour was observed in 47 control rats (Saknyn & Blokhin, 1978). [The Working Group noted that it was not specified whether control rats were untreated or were treated with the vehicle.]

(v) *Intravenous administration*

Rat: A group of 61 male and 60 female Sprague-Dawley rats, eight to nine weeks of age, received six injections of 9 mg/kg bw nickel carbonyl (as Ni) at two- to four-week intervals and were observed for life. Nineteen animals developed malignancies, six of which were undifferentiated sarcomas and three, fibrosarcomas at various sites; the other tumours were single carcinomas of the liver, kidney and mammary gland, one haemangioendothelioma, one undifferentiated leukaemia and five pulmonary lymphomas. Two pulmonary lymphomas developed in 15 male and 32 female sham-injected controls. The difference in total tumour incidence was significant ($p < 0.05$) (Lau *et al.*, 1972).

(vi) *Intrarenal administration*

Groups of male fischer rats [initial number unspecified], approximately eight weeks old, received intrarenal injections of 7 mg nickel as one of several nickel compounds in 0.1 or 0.2 ml saline solution or in glycerol:distilled water (1:1, v/v) in each pole of the right kidney and were observed for two years after treatment. The incidence of renal cancer was significantly elevated in the groups treated with nickel sulfarsenide (3/15 sarcomas) but not in those treated with nickel arsenide (1/20 renal-cell carcinoma), nickel selenide (1/12 sarcoma), nickel subselenide (2/23 sarcomas), nickel telluride (0/19), nickel subarsenides (tetragonal and hexagonal; 0/15 and 0/17), nickel antimonide (0/20) or nickel titanate (0/19). No local tumour developed in vehicle controls (Sunderman *et al.*, 1984b).

The experiments described in section 3.1 are summarized in Table 24.

Table 24. Summary of studies used to evaluate the carcinogenicity to experimental animals of metallic nickel and nickel compounds

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Metallic nickel powder and nickel alloys				
Cr/Ni stainless steel	Intratracheal	Hamster (60)	36 mg, no local tumour 108 mg, no local tumour	Muhle *et al.* (1990)
Metallic nickel powder	Inhalation	Mouse (20)	No lung tumour	Hueper (1958)
Metallic nickel powder	Inhalation	Rat (160)	Benign lung neoplasms	Hueper (1958)
Metallic nickel powder (plus sulfur dioxide)	Inhalation	Rat (120)	0/46 lung tumour	Hueper & Payne (1962)
Metallic nickel powder	Inhalation	Guinea-pig (42)	1/23 intra-alveolar carcinoma, 1/23 metastasis of adenocarcinoma	Hueper (1958)
Metallic nickel powder	Intratracheal	Rat (80)	10. × 0.9 mg, 8/32 lung tumours [$p < 0.05$] 20 × 0.3 mg, 10/39 lung tumours [$p < 0.05$] Controls, 0/40 lung tumour	Pott *et al.* (1987)
Metallic nickel powder	Intratracheal	Hamster (100 per group)	10 mg, 1% local tumours 20 mg, 8% local tumours 40 mg, 12% local tumours 4 × 20 mg, 10% local tumours	Ivankovic *et al.* (1987)
Metallic nickel powder	Intratracheal	Hamster (60)	1/56 lung tumour	Muhle *et al.* (1990)
Metallic nickel powder	Intrapleural	Rat (25)	4/12 local sarcomas *vs* 0/70 in controls [$p < 0.01$]	Hueper (1952)
Metallic nickel powder	Intrapleural	Rat (10)	2/10 mesotheliomas *vs* 0/20 in controls	Furst *et al.* (1973)
Metallic nickel powder	Subcutaneous	Rat (10)	5/10 local tumours	Mitchell *et al.* (1960)
Metallic nickel powder	Intramuscular	Rat (10)	10/10 local tumours *vs* 0 in controls	Heath & Daniel (1964)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Metallic nickel powder	Intramuscular	Rat (50)	38/50 local tumours *vs* 0 in controls	Furst & Schlauder (1971)
Metallic nickel powder	Intramuscular	Rat (20)	3.6 mg, 0/10 local tumours 14.4 mg, 2/9 local tumours Controls, 0/20 local tumours	Sunderman & Maenza (1976)
Metallic nickel powder	Intramuscular	Rat (20)	17/20 local tumours *vs* 0/56 in controls	Berry *et al.* (1984)
Metallic nickel powder	Intramuscular	Rat (20)	13/20 local tumours *vs* 0/44 in controls	Sunderman (1984)
Metallic nickel powder	Intramuscular	Rat (40)	14/30 local tumours *vs* 0/60 in controls	Judde *et al.* (1987)
Metallic nickel powder	Intramuscular	Hamster (50)	2/50 local tumours *vs* 0/50 in controls	Furst & Schlauder (1971)
Metallic nickel powder	Intraperitoneal	Rat	30–50% local tumours *vs* none in controls	Furst & Cassetta (1973)
Metallic nickel powder	Intraperitoneal	Rat (50)	46/48 abdominal tumours	Pott *et al.* (1987)
Metallic nickel powder	Intraperitoneal	Rat	6 mg, 4/34 local tumours 2×6 mg, 5/34 local tumours 25×1 mg, 25/35 local tumours	Pott *et al.* (1990)
Metallic nickel powder	Intravenous	Mouse (25)	No tumour	Hueper (1955)
Metallic nickel powder	Intravenous	Rat (25)	7/25 local tumours	Hueper (1955)
Metallic nickel powder	Intrarenal	Rat (20)	No local tumour	Jasmin & Riopelle (1976)
Metallic nickel powder	Intrarenal	Rat	No local tumour	Sunderman *et al.* (1984b)
Metallic nickel powder	Subperiosteal	Rat (20)	11/20 local tumours	Berry *et al.* (1984)
Metallic nickel powder	Intrafemoral	Rat (20)	9/20 local tumours	Berry *et al.* (1984)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel alloy: 26.8%, Ni, 16.2% Cr, 39.2% Fe, 0.04% Co	Intratracheal	Hamster (100 per group)	10 mg, no local tumour 20 mg, no local tumour 40 mg, no local tumour 4×20 mg, no local tumour	Ivankovic *et al.* (1987)
Nickel alloy: 66.5%, Ni, 12.8% Cr, 6.5% Fe, 0.2% Co	Intratracheal	Hamster (100 per group)	10 mg, 1% local tumours 20 mg, 8% local tumours 40 mg, 12% local tumours 4×20 mg, 10% local tumours	Ivankovic *et al.* (1987)
Nickel–gallium alloy (60% Ni)	Subcutaneous	Rat (10)	9/10 local tumours	Mitchell *et al.* (1960)
Nickel–iron alloy ($NiFe_{1-6}$)	Intramuscular	Rat (16)	0/16 local tumours	Sunderman (1984)
Nickel–iron alloy ($NiFe_{1-6}$)	Intrarenal	Rat	1/14 renal cancers *vs* 0/46 controls	Sunderman *et al.* (1984b)
Nickel alloy (50% Ni)	Intraperitoneal	Rat	50 mg, 8/35 local tumours 3×50 mg, 13/35 local tumours	Pott *et al.* (1989, 1990)
Nickel alloy (29% Ni)	Intraperitoneal	Rat	50 mg, 2/33 local tumours 2×50 mg, 1/36 local tumours	Pott *et al.* (1989, 1990)
Nickel alloy (66% Ni)	Intraperitoneal	Rat	50 mg, 12/35 local tumours 3×50 mg, 22/33 local tumours	Pott *et al.* (1989, 1990)
Pentlandite	Intratracheal	Hamster (60)	1/60 local tumour	Muhle *et al.* (1990)
Nickel oxides and hydroxides				
Nickel monoxide (green)	Inhalation	Rat (6, 8)	8 mg/m³, 1/8 lung tumour 0.6 mg/m³, 0/6 lung tumour	Horie *et al.* (1985)
Nickel monoxide	Inhalation	Rat (40, 20)	0.06 mg/m³, no tumour 0.2 mg/m³, no tumour	Glaser *et al.* (1986)
Nickel monoxide	Inhalation	Hamster (51)	1/51 osteosarcoma	Wehner *et al.* (1975, 1979)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel monoxide	Intrapleural	Rat (32)	31/32 local tumours *vs* 0/32 in controls	Skaug *et al.* (1985)
Nickel monoxide	Intratracheal	Rat	10×5 mg, 10/37 lung tumours 10×15 mg, 12/38 lung tumours controls, 0/40	Pott *et al.* (1987)
Nickel monoxide	Intratracheal	Hamster (50)	1/49 lung tumours *vs* 4/50 in controls	Farrell & Davis (1974)
Nickel monoxide	Intramuscular	Mouse (50, 52)	33/50 and 23/52 local tumours	Gilman (1962)
Nickel monoxide	Intramuscular	Rat (32)	21/32 local tumorus	Gilman (1962)
Nickel monoxide	Intramuscular	Rat (20)	2/20 local tumours	Gilman (1966)
Nickel monoxide	Intramuscular	Rat (20)	No local tumour	Sosiński (1975)
Nickel monoxide	Intramuscular	Rat (15)	14/15 local tumours	Sunderman & McCully (1983)
Nickel monoxide	Intramuscular	Rat (20)	0/20 local tumour	Berry *et al.* (1984)
Nickel monoxide	Subperiosteal	Rat (20)	0/20 local tumour	Berry *et al.* (1984)
Nickel monoxide	Intraperitoneal	Rat (50)	46/47 local tumours	Pott *et al.* (1987)
Nickel monoxide	Intraperitoneal	Rat	25 mg, 12/34 local tumours 100 mg, 15/36 local tumours	Pott *et al.* (1989, 1990)
Nickel monoxide (green)	Intrarenal	Rat (12)	0/12 local tumour	Sunderman *et al.* (1984b)
Nickel hydroxide	Intramuscular	Rat	15/20 local tumours	Gilman (1966)
Nickel hydroxide	Intramuscular	Rat (3 x 20)	Dried gel: 5/19 local tumours Crystalline: 3/20 local tumours Colloidal: 0/13 local tumour	Kasprzak *et al.* (1983)
Nickel trioxide	Intramuscular	Rat (10)	0/10 local tumour	Judde *et al.* (1987)
Nickel trioxide	Intracerebral	Rat (20)	3/20 local tumours	Sosiński (1975)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel sulfides				
Nickel disulfide	Intramuscular	Rat	12/14 local tumours	Sunderman (1984)
Nickel disulfide	Intrarenal	Rat	2/10 local tumours	Sunderman *et al.* (1984b)
Nickel sulfide (amorphous)	Intramuscular	Rat (10 per group)	5.6 mg, no local tumour 22.4 mg, no local tumour	Sunderman & Maenza (1976)
β-Nickel sulfide	Intramuscular	Rat	14/14 local tumours	Sunderman (1984)
Nickel sulfide (amorphous)	Intramuscular	Rat	3/25 local tumours	Sunderman (1984)
Nickel sulfide	Intrarenal	Rat (18)	0/18 local tumour	Jasmin & Riopelle (1976)
β-Nickel sulfide	Intrarenal	Rat	8/14 local tumours	Sunderman *et al.* (1984b)
Nickel sulfide (amorphous)	Intrarenal	Rat	0/15 local tumour	Sunderman *et al.* (1984b)
Nickel subsulfide	Inhalation	Rat (226)	14/208 malignant lung tumours; 15/208 benign lung tumours	Ottolenghi *et al.* (1974)
Nickel subsulfide	Intratracheal	Mouse (100)	No increase in lung tumours	Fisher *et al.* (1986)
Nickel subsulfide	Intratracheal	Rat	0.94 mg: 7/47 lung tumours 1.88 mg: 13/45 lung tumours 3.75 mg: 12/40 lung tumours	Pott *et al.* (1987)
α-Nickel subsulfide	Intratracheal	Hamster (62)	0/62 lung tumour	Muhle *et al.* (1990)
Nickel subsulfide	Intrapleural	Rat (32)	28/32 local tumours	Skaug *et al.* (1985)
Nickel subsulfide	Subcutaneous	Mouse (20)	5 mg, 4/8 local tumours 10 mg, 7/8 local tumours	Oskarsson *et al.* (1979)
Nickel subsulfide	Subcutaneous	Rat (40 per group)	3.3 mg, 37/39 local tumours 10 mg, 37/40 local tumours	Mason (1972)
Nickel subsulfide	Subcutaneous	Rat (20)	18/19 local tumours	Shibata *et al.* (1989)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel subsulfide	Intramuscular	Mouse (45, 18)	Swiss, 27/45 local tumours C3H, 9/18 local tumours	Gilman (1962)
Nickel subsulfide	Intramuscular	Mice (20)	5 mg, 4/8 local tumours 10 mg, 4/8 local tumours	Oskarsson *et al.* (1979)
Nickel subsulfide	Intramuscular	Mouse (10, 10)	C57Bl6, 5/10 local tumours DBA/2, 6/10 local tumours	Sunderman (1983b)
Nickel subsulfide	Intramuscular	Rat (32)	25/28 local tumours	Gilman (1962)
Nickel subsulfide	Intramuscular	Rat (20)	10 mg powder, 19/20 local tumours 500 mg fragments, 5/7 local tumours 500 mg discs, 14/17 local tumours 10 mg diffusion chamber, 14/17 local tumours controls, 1/19 local tumour	Gilman & Herchen (1963)
Nickel subsulfide (disc)	Intramuscular	Rat (groups of 15)	4/10 local tumours with removal of disc after 64 days 7/10 local tumours with removal of disc after 128 days 10/10 local tumours with removal of disc after 206 days	Herchen & Gilman (1964)
Nickel subsulfide	Intramuscular	Rat (30, 27)	NIH black, 28/28 local tumours Hooded, 14/23 local tumours	Daniel (1966)
Nickel subsulfide	Intramuscular	Rat (40 per group)	3.3 mg, 38/39 local tumours 10 mg, 34/40 local tumours	Mason (1972)
Nickel subsulfide	Intramuscular	Rat (10 per group)	5 mg, 8/20 local tumours 20 mg, 9/9 local tumours	Sunderman & Maenza (1976)
Nickel subsulfide	Intramuscular	Rat (63,20)	Fischer, 59/63 local tumours Hooded, 11/20 local tumours	Yamashiro *et al.* (1980)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel subsulfide	Intramuscular	Rats (groups of 30)	0.6 mg, 7/30 local tumours 1.2 mg, 23/30 local tumours 2.5 mg, 28/30 local tumours 5 mg, 29/30 local tumours	Sunderman *et al.* (1976)
Nickel subsulfide	Intramuscular	Rat	0.63 mg, 7/29 local tumours 20 mg, 9/9 local tumours	Sunderman (1981)
α-Nickel subsulfide	Intramuscular	Rat	9/9 local tumours	Sunderman (1984)
Nickel subsulfide	Intramuscular	Rat (20)	10/20 local tumours	Berry *et al.* (1984)
Nickel subsulfide	Intramuscular	Rat (100)	2/100 local tumours	Judde *et al.* (1987)
Nickel subsulfide	Intramuscular	Hamster (15, 17)	5 mg, 4/15 local tumours 10 mg, 12/17 local tumours controls, 0/14 local tumour	Sunderman (1983a)
Nickel subsulfide	Intramuscular	Rabbit	16 local tumours	Hildebrand & Biserte (1979a,b)
α-Nickel subsulfide	Intramuscular	Rabbit (4)	0/4 local tumour	Sunderman (1983a)
Nickel subsulfide	Intramuscular	Rat (20)	19/20 local tumours	Shibata *et al.* (1989)
α-Nickel subsulfide	Topical	Hamster (6–7, 13–15)	54 mg total, 0/6 local tumour; 108 mg total, 0/7 local tumour; 540 mg total, 0/15 local tumour; 1080 mg total, 0/13 local tumour	Sunderman (1983b)
Nickel subsulfide	Intraperitoneal	Rat (37)	9/37 local tumours	Gilman (1966)
Nickel subsulfide	Intraperitoneal	Rat (50)	27/42 local tumours	Pott *et al.* (1987)
Nickel subsulfide	Intraperitoneal	Rat	6 mg, 20/36 local tumours 12 mg, 23/35 local tumours 25 mg, 25/34 local tumours	Pott *et al.* (1989, 1990)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel subsulfide	Subperiosteal	Rat (20)	0/20 local tumour	Berry *et al.* (1984)
Nickel subsulfide	Intrafemoral	Rat (20)	10/20 local tumours	Berry *et al.* (1984)
Nickel subsulfide	Intrarenal	Rat (16/24)	In glycerin, 7/16 local tumours In saline, 11/24 local tumours	Jasmin & Riopelle (1976)
α-Nickel subsulfide	Intrarenal	Rat (11–32)	Wistar Lewis, 7/11 local tumours NIH black, 6/12 local tumours Fischer 344, 9/32 local tumours Long-Evans, 0/12 local tumour	Sunderman *et al.* (1979a)
Nickel subsulfide	Intratesticular	Rat (19)	16/19 local tumours	Damjanov *et al.* (1978)
Nickel subsulfide	Intraocular	Rat (15)	14/15 local tumours	Albert *et al.* (1980); Sunderman (1983b)
Nickel subsulfide	Intraocular	Salamander (8)	7/8 local tumours	Okamoto (1987)
Nickel subsulfide	Transplacental	Rat (8)	No difference in tumour incidence	Sunderman *et al.* (1981)
Nickel subsulfide	Pellet implantation into subcutaneous implanted tracheal grafts	Rat (60, 64)	5 mg, 9/60 local tumours 15 mg, 45/64 local tumours	Yarita & Nettesheim (1978)
Nickel subsulfide	Intra-articular	Rat (20)	16/19 local tumours	Shibata *et al.* (1989)
Nickel subsulfide	Intra-fat	Rat (20)	9/20 local tumours	Shibata *et al.* (1989)
Nickel ferrosulfide	Intramuscular	Rat	15/15 local tumours	Sunderman (1984)
Nickel ferrosulfide	Intrarenal	Rat	1/12 local tumour	Sunderman *et al* (1984b)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel salts				
Basic nickel carbonate tetrahydrate	Intraperitoneal	Rat	25 mg, 1/35 lung tumours *vs* 1/33 in controls 50 mg, 3/33 lung tumours *vs* 1/33 in controls	Pott *et al.* (1989, 1990)
Nickel acetate	Intramuscular	Rat (35)	1/35 local tumour	Payne (1964)
Nickel acetate	Intraperitoneal	Mouse (3 x 20)	72 mg, 8/18 lung tumours 180 mg, 7/14 lung tumours 360 mg, 12/19 lung tumours	Stoner *et al.* (1976)
Nickel acetate tetrahydrate	Intraperitoneal	Mouse (30)	1.50 lung tumours/animal Controls, 0.32 lung tumours/animal	Poirier *et al.* (1984)
Nickel acetate tetrahydrate	Intraperitoneal	Rat	25 mg, 3/35 lung tumours *vs* 1/33 in controls 50 mg, 5/31 lung tumours *vs* 1/33 in controls	Pott *et al.* (1989, 1990)
Nickel ammonium sulfate	Intramuscular	Rat (35)	0/35 local tumour	Payne (1964)
Nickel carbonate	Intramuscular	Rat (35)	6/35 local tumours	Payne (1964)
Nickel chloride	Intramuscular	Rat (35)	0/35 local tumour	Payne (1964)
Nickel chloride hexahydrate	Intraperitoneal	Rat	4/32 lung tumours *vs* 1/33 in controls	Pott *et al.* (1989, 1990)
Nickel chromate	Intramuscular	Rat (16)	1/16 local tumour	Sunderman (1984)
Nickel fluoride	Intramuscular	Rat (20)	3/18 local tumours	Gilman (1966)
Nickel sulfate	Intramuscular	Rat (35)	1/35 local tumour	Payne (1964)
Nickel sulfate	Intramuscular	Rat (20)	0/20 local tumour	Gilman (1966)
Nickel sulfate	Intramuscular	Rat (20)	0/20 local tumour	Kasprzak *et al.* (1983)
Nickel sulfate hexahydrate	Intramuscular	Rat (32)	0/32 local tumour	Gilman (1962)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel sulfate heptahydrate	Intraperitoneal	Rat	6/30 lung tumours *vs* 1/33 in controls	Pott *et al.* (1989, 1990)
Other nickel compounds				
Ferronickel alloy	Intramuscular	Rat	No local tumour	Sunderman & McCully (1983)
Nickel antimonide	Intramuscular	Rat	17/29 *vs* 0/40 control ($p < 0.05$)	Sunderman & McCully (1983)
Nickel antimonide	Intrarenal	Rat	0/20 local tumour	Sunderman *et al.* (1984b)
Nickel arsenide	Intramuscular	Rat	No local tumour	Sunderman & McCully (1983)
Nickel arsenide	Intrarenal	Rat	1/20 local tumour	Sunderman *et al.* (1984b)
Nickel arsenide hexagonal	Intramuscular	Rat	17/20 *vs* 0/40 controls ($p < 0.05$)	Sunderman & McCully (1983)
Nickel arsenide hexagonal	Intrarenal	Rat	0/17 local tumour	Sunderman *et al.* (1984b)
Nickel arsenide tetragonal	Intramuscular	Rat	8/16 *vs* 0/40 control ($p < 0.05$)	Sunderman & McCully (1983)
Nickel arsenide tetragonal	Intrarenal	Rat	0/15 local tumour	Sunderman *et al.* (1984b)
Nickel carbonyl	Inhalation	Rat (64, 32, 80)	30 mg/m³ for 32 weeks: 1/64 pulmonary tumour 60 mg/m³ for 32 weeks: 1/32 pulmonary tumour 250 mg/m³ once: 1/80 pulmonary tumour	Sunderman *et al.* (1957, 1959)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel carbonyl	Inhalation	Rat	1/71 lung tumour *vs* 0/32 control	Sunderman & Donnelly (1965)
Nickel carbonyl	Intravenous	Rat (121)	19/120 lung tumours	Lau *et al.* (1972)
Nickel-enriched fly ash	Inhalation	Hamster (102)	No significant difference	Wehner *et al.* (1981, 1984)
Nickelocene	Intramuscular	Rat (50)	144 mg, 18/50 local tumours 300 mg, 21/50 local tumours	Furst & Schlauder (1971)
Nickelocene	Intramuscular	Hamster (50)	8×5 mg, 0/50 local tumour 25 mg, 4/29 local tumours	Furst & Schlauder (1971)
Nickel monoxide dust	Intratracheal	Rat (26)	1/26 lung tumour *vs* 0/47 control	Saknyn & Blokhin (1978)
Nickel selenide	Intramuscular	Rat	8/16 local tumours	Sunderman (1984)
Nickel selenide	Intramuscular	Rat	1/12 local tumour *vs* 0/79 control	Sunderman *et al.* (1984b)
Nickel subselenide	Intramuscular	Rat	21/23 local tumours	Sunderman (1984)
Nickel subselenide	Intrarenal	Rat	2/23 local tumours *vs* 0/79 control	Sunderman *et al.* (1984b)
Nickel sulfarsenide	Intramuscular	Rat	14/16 *vs* 0/40 control ($p < 0.05$)	Sunderman & McCully (1983)
Nickel sulfarsenide	Intrarenal	Rat	3/15 local tumours *vs* 0/79 control	Sunderman *et al* (1984b)
Nickel telluride	Intramuscular	Rat	14/26 *vs* 0/40 control ($p < 0.05$)	Sunderman & McCully (1983)
Nickel telluride	Intrarenal	Rat	0/19 local tumour	Sunderman *et al.* (1984b)
Nickel titanate	Intramuscular	Rat	No local tumour	Sunderman & McCully (1983)

Table 24 (contd)

Compound	Route	Species (No. at start)	Tumour incidence (no. of animals with tumours/effective number)	Reference
Nickel titanate	Intrarenal	Rat	0/19 local tumour	Sunderman *et al.* (1984b)
Refinery dust	Inhalation	Rat (5)	1/5 lung tumour *vs* 0/47 control	Saknyn & Blokhin (1978)
Refinery dust	Intramuscular	Mouse (40)	20/36 local tumours *vs* 0/48 control ($p < 0.01$)	Gilman & Ruckerbauer (1962)
Refinery dust	Intramuscular	Rat (35)	Dust, 19/27 local tumours Washed dust, 20/28 local tumours Controls, 0/30 local tumour	Gilman & Ruckerbauer (1962)
Refinery dust	Intraperitoneal	Rat (16, 23)	Dust 1, 3/16 local tumours Dust 2, 3/23 local tumours Controls, 0/47 local tumour	Saknyn & Blokhin (1978)

3.2 Other relevant data in experimental systems

(*a*) *Absorption, distribution, excretion and metabolism*

The results of studies on absorption, distribution, excretion, and metabolism of nickel compounds have been reviewed and/or summarized in several publications (National Research Council, 1975; Sunderman, 1977; Kasprzak, 1978; Bencko, 1983; Mushak, 1984; Sarkar, 1984; Fairhurst & Illing, 1987; Kasprzak, 1987; Sunderman, 1988; Maibach & Menné, 1989).

(i) *Nickel oxides and hydroxides*

Male Wistar rats were exposed to 0.4-70 mg/m^3 (0.6-4-μm particles) nickel monoxide aerosols for 6-7 h per day on five days per week for a maximum of three months. The clearance rate of nickel monoxide from the lung after a one-month exposure to 0.6-8 mg/m^3 (1.2-μm particles) was estimated to be about 100 μg per year. The exposure did not increase background nickel levels in organs other than the lung (Kodama *et al.*, 1985).

Electron microscopic examination of the lungs of male Wistar rats exposed to nickel monoxide aerosols (0.6-8 mg/m^3; 1.2- or 2.2-μm particles) for a total of 140-216 h showed that the particles were trapped mainly by alveolar macrophages. One year after termination of exposure, the particles were distributed in the alveoli, hilar lymphoid apparatus and terminal bronchioli. Some nickel monoxide particles were present within the lysosomes of macrophages (Horie *et al.*, 1985).

Female Wistar rats were given a single intratracheal injection of black nickel monoxide, prepared by heating nickel hydroxide at 250°C for 45 min (final product containing a mixture of nickel monoxide and nickel hydroxide; >90% insoluble in water; particles, 3.7 μm or less in diameter) in a normal saline suspension (100 nmol [7.5 μg] nickel monoxide in 0.2 ml). The highest concentrations of nickel were seen in the lungs and mediastinal lymph nodes, followed by the heart, femur, duodenum, kidney, pancreas, ovaries, spleen, blood and other tissues. Following injection, the concentration of nickel in the lung decreased at a much slower rate than in other tissues. By the third day after injection of nickel monoxide, about 17% of the nickel was excreted with the faeces and about 16% in the urine. By 90 days, about 60% of the dose of nickel had been excreted, half of it in the urine. The overall pattern indicates a partial transfer of nickel from lung to the mediastinal lymph nodes and slow solubilization of this product in tissue fluids (English *et al.*, 1981).

(ii) *Nickel subsulfide*

After intratracheal instillation of 11.7 μg α-^{63}Ni-nickel subsulfide powder (1-66-μm particles) in a normal saline suspension to male strain A/J mice, 38% was cleared from the lungs with a half-time of 1.2 days, while 42% was cleared with a

half-time of 12.4 days; 10% of the dose was retained in the lung 35 days after instillation. The highest amounts of nickel were found in the kidney, followed by blood > liver > femur up to seven days; at 35 days, levels were greatest in kidney, followed by femur > liver > blood; maximal levels occurred 4 h after dosing and decreased rapidly thereafter with biological half-times similar to those in the lung. The urine was the primary excretion pathway; after 35 days, 100% of the nickel dose was recovered in the excreta, 60% of which was in urine (Valentine & Fisher, 1984).

The cumulative eight-week urinary excretion of nickel following intramuscular injection of ^{63}Ni-nickel subsulfide to male Fischer rats (1.2 mg/rat, 1.4-μm particles) was 67%, while faecal excretion during that time was only 7% of the dose. The residual nickel contents at the injection site at 22 and 31 weeks after injection were 13-17% and 13-14% of the dose, respectively. The kinetics of nickel disappearance were described by a three-compartmental model, with pool sizes of 60, 27 and 11% of the dose and half-times of 14, 60 and indefinite number of days, respectively (Sunderman *et al.*, 1976).

α-Nickel subsulfide particles labelled with ^{63}Ni and ^{35}S injected intramuscularly into Fischer rats (Kasprzak, 1974) or intramuscularly and subcutaneously into NMRI mice of each sex (Oskarsson *et al.*, 1979) persisted at the injection site for several months, with a gradual loss of both ^{63}Ni and ^{35}S. In mice, nickel subsulfide was transferred to regional lymph nodes and to the reticuloendothelial cells of the liver and spleen. The presence of ^{63}Ni in the kidney and ^{35}S in the cartilage indicated solubilization of the subsulfide from the site of injection during tumorigenesis. There was no excessive or specific localization of the solubilized ^{63}Ni or ^{35}S in the tumours or in metastases. Most of the radioactivity in the tumours appeared to be associated with dust particles.

Elevated concentrations of nickel were detected in fetuses after intramuscular administration of α-nickel subsulfide to Fischer rats on day 6 of gestation (Sunderman *et al.*, 1978a).

(iii) *Nickel salts*

Intratracheal instillation of nickel chloride (100 nmol[13 μg]/rat) to female Wistar rats resulted in a fast distribution of nickel throughout the body, followed by rapid clearance. During the first six days after injection, over 60% of the dose was excreted in the urine and approximately 5% in faeces; after 90 days, these amounts had increased only slightly, to 64% and 6%, respectively (English *et al.*, 1981). Similar distribution and excretion patterns were observed after intratracheal injection of nickel chloride (1.27 μg/rat Ni) to male Sprague-Dawley rats (Carvalho & Ziemer, 1982).

Pulmonary clearance and excretion of nickel following intratracheal instillation of nickel sulfate at doses of 17, 190 or 1800 nmol [1, 11 or 106 μg] Ni per rat to

Fischer 344 rats appeared to depend on the dose. At periods up to four days after instillation, lungs, trachea, larynx, kidney and urinary bladder contained the highest concentrations of nickel. The half-time for urinary excretion (the predominant route of excretion) varied from 23 h for the lowest dose to 4.6 h for the highest. Faecal excretion accounted for 30% (17- and 190-nmol doses) and 13% (1800-nmol) of the dose. The long-term half-time of nickel clearance from the lung varied from 21 h at the highest dose to 36 h at the lowest dose (Medinsky *et al.*, 1987).

In male Sprague-Dawley rats exposed to nickel chloride aerosols (90 μg/m^3 Ni; 0.7-0.9-μm particles) for 2 h per day for 14 days, the nickel burden in the lung reached a steady level after five days. The maximal clearance velocity was calculated to be 34.6 ng/g.h. These data support the hypothesis of a saturable clearance mechanism for 'soluble nickel' in the lung (Menzel *et al.*, 1987).

After intratracheal administration of 'nickel carbonate' (0.05 mg/mouse Ni) to female Swiss albino mice, most of the dose was eliminated in the urine in about 12 days (Furst & Al-Mahrouq, 1981). [The Working Group noted that the compound tested was most probably basic nickel carbonate.]

After a single intravenous injection of 10 μg nickel as ^{63}Ni-nickel chloride per mouse (albino or brown mice [strains not specified], including pregnant mice), whole-body autoradiography at 30 min showed that nickel persisted in the blood, kidney, urinary bladder, lung, eye and hair follicles; at three weeks, nickel persisted in the lung, central nervous system, kidneys, hair follicles and skin (Bergman *et al.*, 1980). In C57Bl mice, nickel was also localized in the epithelium of the forestomach; in the kidney, it was present in the cortex at sites that probably corresponded to the distal convoluted tubules. Nickel was retained much longer in the lung than in other tissues (Oskarsson & Tjälve, 1979a).

A single intravenous injection of 1 mg/kg bw ^{63}Ni-nickel chloride to male Sprague-Dawley rats resulted in rapid urinary excretion of 87% of the dose in the first day after injection and 90% after four days. Faecal excretion was much lower, up to a total of approximately 3% of the dose after four days (Sunderman & Selin, 1968). Lung and spleen were ranked after kidney as nickel-accumulating organs in Sprague-Dawley rats given an intraperitoneal injection of 82 μg/kg bw ^{63}Ni-nickel chloride (Sarkar, 1980).

The kinetics of nickel metabolism in rats and rabbits after a single intravenous injection of ^{63}Ni-nickel chloride followed a two-compartmental mathematical model, with first-order kinetics of nickel elimination from plasma with half-times of 6 and 50 h for rats and 8 and 83 h for rabbits, respectively, for the two compartments (Onkelinx *et al.*, 1973).

Following a single intraperitoneal injection of ^{63}Ni-nickel chloride to BALB/c mice (100 μCi/mouse), nickel was found to remain in the lung much longer than in

any other tissue (Herlant-Peers *et al.*, 1982). Preferential accumulation of nickel in the lung was also observed in Fischer 344 rats following daily subcutaneous injections of 62.5 or 125 μmol [8.1 or 16.3 mg]/kg bw nickel chloride for up to six weeks (Knight *et al.*, 1988). In contrast, multiple intraperitoneal injections of nickel acetate to male Swiss albino mice (0.5, 0.75 or 1.0 mg/mouse; 10, 20 or 30 daily injections each) resulted in preferential accumulation of nickel in the thymus (Feroz *et al.*, 1976).

Daily oral administration of 2.5 mg nickel sulfate per rat [strain unspecified] for 30 days resulted in accumulation of nickel in trachea > nasopharynx > skull > oesophagus > intestine > skin > liver = spleen > stomach > kidney > lung = brain > heart (Jiachen *et al.*, 1986).

Nickel was taken up from the lumen of male Sprague-Dawley rat jejunum *in vitro* at a rate proportional to the concentration of ^{63}Ni-nickel chloride in the perfusate up to 20 μM [1.2 mg] Ni. At higher concentrations (6 and 12 mg Ni), apparent saturation was approached. Nickel was not retained by the mucosa and showed a very low affinity for metallothionein (Foulkes & McMullen, 1986).

Dermal absorption of 2 or 40 μCi ^{63}Ni-nickel chloride was observed in guinea-pigs. After 1 h, nickel had accumulated in highly keratinized areas, the stratum corneum and hair shafts. Following exposure for 4-48 h, nickel also accumulated in basal and suprabasal epidermal cells. After 4 h, nickel appeared in blood and urine (Lloyd, 1980).

It has been demonstrated in several studies that nickel chloride crosses the placenta in mice (Jacobsen *et al.*, 1978; Lu *et al.*, 1979; Olsen & Jonsen, 1979; Lu *et al.*, 1981; Jasim & Tjälve, 1986) and rats (Sunderman *et al.*, 1977; Mas *et al.*, 1986).

(iv) *Other nickel compounds*

In NMRI mice, high levels of nickel were found in the respiratory tract, brain, spinal cord, heart, diaphragm, adrenal cortex, brown fat, kidney and urinary bladder 5 min to 24 h following inhalation of ^{63}Ni- and ^{14}C-nickel carbonyl at 3.05 g/m^3 Ni for 10 min (Oskarsson & Tjälve, 1979b).

After exposure of rats to nickel carbonyl by inhalation, increased levels of nickel were found predominantly in microsomal and supernatant fractions of the lung and in the microsomal fraction of the liver (Sunderman & Sunderman, 1963).

After an intravenous injection of nickel carbonyl as 22 mg/kg bw Ni to Sprague-Dawley rats, most of the subcellular nickel in liver and lung was bound to supernatant fractions, followed by nuclei and debris, mitochondria and microsomes (Sunderman & Selin, 1968).

Twenty-four hours after an intravenous injection of ^{63}Ni-nickel carbonyl (0.9 mg/kg bw Ni) to NMRI mice, nickel was found to be associated with both particulate and soluble cellular constituents of the lung, liver and kidneys. Radioactivity

was detected in the gel chromatograms of cytosols from lung, kidney and blood serum of treated mice in the void volume and salt volume (Oskarsson & Tjälve, 1979c).

Following intravenous injection of 50 µl/kg bw nickel carbonyl (22 µg/kg bw Ni) to Sprague-Dawley rats, over 38% of the dose was exhaled during 6 h after injection and none after that time. Average total urinary excretion of nickel over four days was 31% (23% within the first 12 h), whereas total faecal excretion was 2.4% and biliary excretion was 0.2%. Total average excretion of nickel in four days was 72%. Most of the remaining nickel carbonyl underwent intracellular decomposition and oxidation to nickel[II] and carbon monoxide. Twenty-four hours after the injection, nickel injected as nickel carbonyl was distributed among organs and tissues, with the highest concentration in lung (Sunderman & Selin, 1968; Kasprzak & Sunderman, 1969).

(*b*) *Dissolution and cellular uptake*

(i) *Metallic nickel and nickel alloys*

Slow dissolution and elimination of finely powdered nickel metal from the muscle injection site was observed in rats. In the local rhabdomyosarcomas that developed, nickel was recovered in the nuclear fraction and mitochondria; little or no nickel was found in the microsomes (Heath & Webb, 1967). The nuclear fraction of nickel is preferentially bound to nucleoli (Webb *et al.*, 1972).

Slow dissolution of metallic nickel occurred when nickel metal powder was incubated at 37°C with horse serum or sterile homogenates of rat muscle, liver, heart or kidney prepared in Tyrode solution. The solubilization may have involved oxygen uptake and was faster for a freshly reduced powder than for an older commercial powder; over 97% of the dissolved nickel became bound to diffusible components of the tissue homogenates (mostly histidine, followed by nucleotides, nucleosides and free bases) (Weinzierl & Webb, 1972).

(ii) *Nickel oxides and hydroxides*

The dissolution half-times of six differently prepared samples of nickel oxide and four samples of nickel-copper oxides in water were longer than 11 years. However, in rat serum and renal cytosol, the half-time dropped to about one year for a low-temperature nickel oxide and to 2.7-7.2 years for three nickel-copper oxides, the rest retaining the $>$ 11-year value. Two preparations of nickel oxide obtained at temperatures $\leq$735°C and all four nickel-copper oxides appeared to be phagocytized by C3H/10T½ cells more actively than the other nickel oxides (Sunderman *et al.*, 1987).

Kasprzak *et al.* (1983) found the half-times for two preparations of nickel hydroxide (air-dried colloidal and crystalline) in an 0.1 M ammonium acetate buffer of

pH 7.4 to be 56 h and 225 h, respectively. Corresponding values in an artificial lung fluid were 360 h and 1870 h, respectively.

(iii) *Nickel sulfides*

The dissolution rate of α-nickel subsulfide depends on the particle size, the presence of oxygen and the dissolving medium (Gilman & Herchen, 1963; Kasprzak & Sunderman, 1977; Dewally & Hildebrand, 1980; Lee *et al.*, 1982).

Both *in vivo* and in cell-free systems *in vitro*, α-nickel subsulfide reacts with oxygen to yield insoluble crystalline β-nickel sulfide and soluble nickel[II] derivatives; β-nickel sulfide also dissolves through oxidation of its sulfur moiety (Kasprzak & Sunderman, 1977; Oskarsson *et al.*, 1979; Dewally & Hildebrand, 1980). It has been suggested that the transformation of nickel subsulfide into β-nickel sulfide under anaerobic conditions in the muscle might be due to reaction with sulfur from sulfhydryl groups in the host organism (Dewally & Hildebrand, 1980).

Particles of crystalline nickel sulfides, α-nickel subsulfide and β-nickel sulfide (<5 μm in diameter, 1-20 μg/ml) were phagocytized by cultured Syrian hamster embryo cells and Chinese hamster CHO cells, while particles of amorphous nickel sulfide were taken up only sparingly by the cells. Pretreatment of Syrian hamster embryo cells with benzo[*a*]pyrene enhanced the uptake of nickel subsulfide. The half-life of the engulfed particles was about 40 h in Syrian hamster cells; they disappeared from the cells through solubilization, and solubilized nickel was detected in the nuclear fraction (Costa & Mollenhauer, 1980a,b; Costa *et al.*, 1981a).

α-Nickel subsulfide and β-nickel sulfide were also incorporated into human embryonic L132 pulmonary cells in culture. β-Nickel sulfide was present within large intracellular vesicles; nickel subsulfide was generally bound to the membranes of intracellular vesicles, to lysosomal structures and to the outer cell membrane (Hildebrand *et al.*, 1985, 1986).

The soluble nickel derived from nickel subsulfide and β-nickel sulfide intracellularly undergoes subcellular distribution that differs from that following entry of nickel from outside the cells (Harnett *et al.*, 1982; Sen & Costa, 1986a). Treatment of cultured Chinese hamster CHO cells with β-nickel sulfide (10 μg/ml, three-day incubation) resulted in binding of nickel to DNA and RNA at a level 300-2000 times higher and to protein at a level 15 times higher than after similar treatment with nickel chloride (Harnett *et al.*, 1982). Cellular uptake of β-nickel sulfide facilitates a specific interaction of nickel with the heterochromatic long arm of the X chromosome of Chinese hamster CHO cells (Sen & Costa, 1986a). Lee *et al.* (1982) found that soluble nickel derived from nickel subsulfide forms an exceptionally stable ternary protein-nickel-DNA complex *in vitro* in the presence of DNA and rat liver microsomes.

(iv) *Nickel salts*

Soluble nickel retained in the tissues of mice becomes bound to particulate and soluble cellular constituents, the distribution depending on the tissue. In lung and liver of NMRI mice, nickel was bound predominantly to a high-molecular-weight protein; in the kidney, it was bound mainly to low-molecular-weight ultrafiltrable ligands. No nickel was bound to metallothionein or superoxide dismutase (Oskarsson & Tjälve, 1979c).

Several nickel-binding proteins were found in lung and liver cytosol of BALB/c mice that were different after incorporation *in vivo* and *in vitro*. The composition and structures of these proteins were not identified (Herlant-Peers *et al.*, 1982).

Intracellular nickel concentrations in the lungs of strain A mice given intraperitoneal injections of nickel acetate were highest in the microsomes, followed by mitochondria, cytosol and nuclei (Kasprzak, 1987).

In blood serum, nickel was sequestered mainly by albumin, which had a high binding capacity for this metal in most species tested, except for dogs and pigs (Callan & Sunderman, 1973). Nickel in human serum is chelated by histidine, serum albumin or both in a ternary complex, although a small fraction is bound to a glycoprotein (Sarkar, 1980; Glennon & Sarkar, 1982).

Less nickel chloride was taken up by Chinese hamster CHO cells than insoluble nickel sulfides; moreover, nickel incorporated from nickel chloride had a much higher affinity for cellular proteins than for DNA or RNA (Harnett *et al.*, 1982). A greater effect on the heterochromatic long arm of the X chromosome was observed when Chinese hamster CHO cells were exposed to nickel-albumin complexes encapsulated in liposomes than to nickel chloride alone (Sen & Costa, 1986a).

Cellular binding and uptake of nickel depend on the hydro- and lipophilic properties of the nickel complexes to which the cells are exposed. Nickel-complexing ligands, L-histidine, human serum albumin, D-penicillamine and ethylenediaminetetraacetic acid, which form hydrophilic nickel complexes, inhibited the uptake of nickel by rabbit alveolar macrophages, human B-lymphoblasts and human erythrocytes. The same ligands also sequestered nickel from nickel-preloaded cells. Diethyldithiocarbamate, however, which forms a lipophilic nickel complex, enhanced the cellular uptake of nickel and prevented its removal from nickel-preloaded cells. It also induced transfer of nickel in a cell lysate from the cytosol to the residual pellet (Nieboer *et al.*, 1984b). Sodium pyridinethione, which forms a lipophilic nickel complex, behaved similarly (Jasim & Tjälve, 1986).

Nickel applied to rat liver and kidney nuclei as nickel chloride bound in a dose-related manner to the chromatin and as to polynucleosomes and to the DNA molecule. In the nuclear chromatin, nickel was associated with both the DNA and histone and non-histone proteins; a ternary nickel-DNA-protein complex more

stable than binary nickel-DNA complexes was identified (Ciccarelli & Wetterhahn, 1985).

Calf thymus DNA appeared to have more than two types of binding site for nickel; DNA phosphate moieties were identified as having the highest affinity for nickel (Kasprzak *et al.*, 1986).

(v) *Other nickel compounds*

'Nickel carbonate' particles were actively phagocytized by human embryonal lung epithelial cells L132 in culture and showed an increased affinity for cytoplasmic and cell membranes (Hildebrand *et al.*, 1986). [The Working Group noted that the compound tested was most probably basic nickel carbonate.]

Following an intraperitoneal injection of 'nickel carbonate' to male Sprague-Dawley rats, nickel was found to be associated with liver and kidney nuclear DNA as early as 3 h after injection, with a further increase by 20 h. The nickel concentration in kidney DNA was five to six times higher than that in liver. Significant differences were found in the distribution of nickel between nucleic acids and associated proteins in DNA samples extracted from kidney and liver (Ciccarelli & Wetterhahn, 1984a,b). [The Working Group noted that the compound tested was most probably basic nickel carbonate.]

Sunderman *et al.* (1984b) determined dissolution half-times in rat serum and renal cytosol and phagocytic indices in peritoneal macrophages *in vitro* of various water-insoluble nickel derivatives, including nickel selenide, nickel subselenide, nickel telluride, nickel sulfarsenide, nickel arsenide, nickel arsenide tetragonal, nickel arsenide hexagonal, nickel antimonide, nickel ferrosulfide matte, a ferronickel alloy ($NiFe_{1-6}$) and nickel titanate. No correlation was found between those two parameters and the carcinogenic activity of the tested compounds in the muscle of Fischer 344 rats.

(c) *Interactions*

Parenteral administration of soluble nickel salts induced changes in the tissue distribution of other metal ions (Whanger, 1973; Nielsen, 1980; Chmielnicka *et al.*, 1982; Nieboer *et al.*, 1984b; Nielsen *et al.*, 1984).

Several physiological divalent cations appeared to affect nickel metabolism. Thus, manganese decreased the proportion of ultrafiltrable nickel constituents of muscle homogenates; the gross muscle uptake and excretion of nickel were not affected. Metallic manganese dust also inhibited the dissolution rate of nickel subsulfide in rat serum, serum ultrafiltrate and water (Sunderman *et al.*, 1976). Manganese dust reduced the phagocytosis of nickel subsulfide particles by Syrian hamster embryo cells *in vitro* (Costa *et al.*, 1981a). Magnesium decreased the uptake of nickel by pulmonary nuclei and cytosol of strain A mice and decreased nickel uptake by

lung, kidney and liver of Fischer 344 rats (Kasprzak *et al.*, 1987). Both manganese and magnesium strongly antagonized the binding of nickel to the phosphate groups of calf thymus DNA *in vitro*, while copper, which did not inhibit nickel carcinogenesis, was a much weaker antagonist (Kasprzak *et al.*, 1986).

Nickel that accumulated in mouse tissues following administration of nickel carbonyl *in vivo* could be displaced from those tissues by treatment *in vitro* with other cations, including H^+, in proportion to their valence; Mg^{2+} and La^{3+} were the most effective (Oskarsson & Tjälve, 1979b).

Certain nickel[II]-peptide complexes in aqueous solution were found to react with ambient oxygen by a facile autocatalytic process in which nickel[III] intermediates played a major role. Such reactions may lead to degradation, e.g., decarboxylation, of the organic ligand (Bossu *et al.*, 1978). Nickel[III] was also identified in a nickel[II]-glycyl-glycyl-*n*-histidine complex, indicating possible redox effects of the nickel[III]/nickel[II] redox couple on that protein (Nieboer *et al.*, 1986).

(*d*) *Toxic effects*

The toxicity of nickel and its inorganic compounds has been reviewed (US Environmental Protection Agency, 1986; Fairhurst & Illing, 1987; World Health Organization, 1990), and the chemical basis of the biological reactivity of nickel has been discussed (Ciccarelli & Wetterhahn, 1984a; Nieboer *et al.*, 1984b,c).

(i) *Metallic nickel and nickel alloys*

The lungs of male rabbits exposed by inhalation to 1 mg/m^3 nickel metal dust (< 40 μm particles) for 6 h per day on five days per week for three and six months showed two- to three-fold increases in the volume density of alveolar type II cells. The six-month exposure caused focal pneumonia (Johansson *et al.*, 1981; Camner *et al.*, 1984).

Similar changes, resembling alveolar proteinosis, were observed in rabbits after exposure to nickel metal dust by inhalation for four weeks (Camner *et al.*, 1978). After three or six months of exposure at 1 mg/m^3, phagocytic activity *in vitro* was increased upon challenge by *Escherichia coli* (Johansson *et al.*, 1980).

A single intramuscular injection of 20 mg nickel metal dust to male WAG rats resulted in long-lasting suppression of natural killer cell activity in peripheral blood mononuclear cells. Between eight and 18 weeks after the nickel injection, the activity decreased to 50-60% of that in the control rats (Judde *et al.*, 1987).

(ii) *Nickel oxides*

Exposure of female Wistar rats by inhalation to nickel monoxide aerosols (generated at 550°C from nickel acetate) at concentrations of 200, 400 and 800 μg/m^3 for 24 h per day for 120 days resulted in a significant, dose-related reduction in growth

rate, decreased kidney and liver weights and erythrocyte count, decreased activity of serum alkaline phosphatase, increased wet lung weight and leukocyte count and increased mean erythrocyte cell volume (Weischer *et al.*, 1980a,b).

Male Wistar rats exposed continuously to nickel monoxide (generated at 550°C from nickel acetate) aerosols at 50 μg/m³ (median particle diameter, 0.35 μm) for 15 weeks showed no significant difference in the overall ability of the lungs to clear ferrous oxide up to day 7. After that time, lung clearance in nickel oxide-exposed rats decreased significantly. The half life of ferrous oxide clearance after day 6 was 58 days for control rats and 520 days for nickel oxide-exposed rats; in excised lungs, the values were 56 and 74 days, respectively (Oberdoerster & Hochrainer, 1980).

An increase in lung weight (six-fold) and alveolar proteinosis were observed in male Wistar rats that died during life-time exposure to an aerosol of nickel monoxide (produced by pyrolysis of nickel acetate [probably at 550°C] [particle size unspecified]) at 60 or 200 μg/m³, 23 h per day, seven days per week. With longer exposures, marked accumulation of macrophages and focal septal fibrosis were also observed (Takenaka *et al.*, 1985).

No significant histopathological change was found in male Wistar rats exposed to green nickel oxide (0.6 μm particles) for up to 12 months at 0.3 or 1.2 mg/m³, 7 h per day on five days per week (Tanaka *et al.*, 1988).

No mortality was observed following exposure by inhalation of Fischer 344/N rats and $B6C3F_1$ mice to nickel monoxide (formed at 1350°C; 3 μm particles) at 0.9-24 mg/m³ Ni for 6 h/day on five days per week for 12 days. Lung inflammation and hyperplasia of alveolar macrophages occurred primarily at the highest exposure concentration in both species; generally, the lung lesions in mice were less severe than those in rats. Atrophy of the olfactory epithelium was seen only in two rats at the highest dose, while atrophy of the thymus and hyperplasia of the lymph nodes were seen in both rats and mice exposed to the highest concentrations (Dunnick *et al.*, 1988).

In Syrian golden hamsters, life-time inhalation of 53 mg/m³ nickel monoxide ([unspecified] 0.3 μm particles) for 7 h per day resulted in emphysema in animals that died early in the experiment. Other lung effects included interstitial pneumonitis and diffuse granulomatous pneumonia, fibrosis of alveolar septa, bronchiolar (basal-cell) hyperplasia, bronchiolization of alveolar epithelium, squamous metaplasia and emphysema and/or atelectasia of various degrees (Wehner *et al.*, 1975).

The median lethal concentration for rat macrophages exposed *in vitro* to green nickel monoxide exceeded 12 μmol (708 μg)/ml Ni. The LC_{50} for canine macrophages was 3.9 μmol (230 μg)/ml Ni as nickel monoxide for 20 h. Nickel monoxide was far less toxic to macrophages than nickel sulfate, nickel chloride or nickel

subsulfide (Benson *et al.*, 1986a). The toxicity of six different preparations of nickel monoxide calcined at temperatures of < 650-1045 °C and four mixed nickel-copper oxides was tested *in vitro* on alveolar macrophages of beagle dogs, Fischer 344 rats and $B6C3F_1$ mice. Nickel oxides were less toxic to the macrophages than were the nickel-copper oxides; the toxicity of the nickel-copper oxides increased with increasing copper content. Generally, dog macrophages were more sensitive to the oxides than mouse and rat macrophages (Benson *et al.*, 1988a).

The ability of the same oxides to stimulate erythropoiesis in Fischer 344 rats correlated well with their cell transforming ability in Syrian hamster embryo cells (see also genetic and related effects; Sunderman *et al.*, 1987).

(iii) *Nickel sulfides*

The LD_{50} after a single instillation in $B6C3F_1$ mice of nickel subsulfide (particle size, < 2 μm) in a normal saline suspension was 4 mg/kg bw (Fisher *et al.*, 1986).

Acute toxic effects of nickel subsulfide (1.8 μm particles) administered intratracheally to male BALB/c mice (12 μg/mouse) included pulmonary haemorrhaging, most evident three days after exposure. The number of polymorphonuclear cells in the pulmonary lavage fluid was increased, whereas the number of macrophages tended to decrease below the control values later (20 h to seven days) after the exposure (Finch *et al.*, 1987).

Alveolitis was observed in Fischer 344 rats following intratracheal instillation of nickel subsulfide as a saline/gelatin suspension (3.2-320 μg/kg bw). The effects closely resembled those of nickel chloride and nickel sulfate at comparable doses of nickel. Pulmonary lesions also included type II cell hyperplasia with epithelialization of alveoli and, in some animals, fibroplasia of the pulmonary interstitium (Benson *et al.*, 1986b).

Chronic active inflammation, fibrosis and alveolar macrophage hyperplasia were observed in Fischer 344 rats and $B6C3F_1$ mice exposed by inhalation to nickel subsulfide (low-temperature form) for 13 weeks (6 h per day, five days per week; 0.11-1.8 mg/m^3 Ni). The toxicity of nickel subsulfide to the lung resembled that of nickel sulfate hexahydrate, and both were more toxic than nickel oxide. Rats were more sensitive than mice (Dunnick *et al.*, 1989).

Administration of nickel subsulfide to female rats as a single intrarenal injection caused pronounced erythrocytosis (Jasmin & Riopelle, 1976; Oskarsson *et al.*, 1981). A single intrarenal injection of nickel subsulfide to male rats also caused an increase in renal haem oxygenase activity; no correlation between the induction of haem oxygenase and erythrocytosis was observed (Sunderman *et al.*, 1983a). Administration of nickel sulfide [probably amorphous] in glycerine or saline into each pole of the kidney of female rats did not induce renal erythropoietic activity (Jasmin & Riopelle, 1976).

Under comparable exposure *in vitro*, beagle dog alveolar macrophages were more sensitive to the toxicity of nickel subsulfide than were those of Fischer 344 rats. Nickel subsulfide appeared to be much more toxic to the macrophages of both species than nickel chloride, nickel sulfate or nickel monoxide (Benson *et al.*, 1986a).

Nickel subsulfide incubated with calf thymus histones in the presence of molecular oxygen caused random polymerization of those proteins; this effect was not produced by soluble nickel acetate (Kasprzak & Bare, 1989).

(iv) *Nickel salts*

The oral LD_{50} of nickel acetate was 350 mg/kg bw in rats and 420 mg/kg bw in mice; the intraperitoneal LD_{50} was 23 mg/kg bw in rats (National Research Council, 1975). The LD_{50} of nickel acetate in ICR mice after an intraperitoneal injection was 97 mg/kg bw in females and 89 mg/kg bw in males at days 3 and 5 for three-week-old animals and 39-54 mg/kg bw in nine- or 14-week-old animals of either sex (Hogan, 1985). With nickel chloride, intraperitoneal LD_{50} values of 6-9.3 mg/kg bw Ni were reported for female Wistar rats (Mas *et al.*, 1985), 11 mg/kg bw rats and 48 mg/kg bw for mice (National Research Council, 1975).

Exposure of $B6C3F_1$ mice and Fischer 344/N rats to nickel sulfate hexahydrate by inhalation for 6 h per day for 12 days (five days per week plus two consecutive days; 0.8-13 mg/m^3 Ni; 2 μm particles) caused death of all mice at concentrations of ≥1.6 mg/m^3 and of some rats at concentrations of 13 mg/m^3. Lesions of the lung and nasal cavity were observed in both mice and rats after exposure to 0.8 mg/m^3 nickel or more; these included necrotizing pneumonia, chronic inflammation and degeneration of the bronchiolar epithelium, atrophy of the olfactory epithelium, and hyperplasia of the bronchial and mediastinal lymph nodes (Benson *et al.*, 1988b; Dunnick *et al.*, 1988).

A single intratracheal instillation of nickel chloride hexahydrate or nickel sulfate hexahydrate to Fischer 344/Cr1 rats (0.01, 0.1 or 1.0 μmol [0.59, 5.9 or 59 μg Ni/rat) caused alveolitis and affected the activities of several enzymes measured in the pulmonary lavage fluid (Benson *et al.*, 1985, 1986b).

Rabbits were exposed to nickel chloride (0.2-0.3 mg/m^3 Ni) for 6 h per day on five days per week for one to eight months. Nodular accumulation of macrophages was seen in lung tissue, and the volume density of alveolar type II cells was elevated. The phagocytic activity of macrophages was normal after one month of exposure but was decreased after three months (Camner *et al.*, 1984; Lundborg & Camner, 1984; Camner *et al.*, 1985).

Exposure of Syrian golden hamsters to a nickel chloride aerosol (100-275 μg/m^3 Ni; <2-μm particles) for 2 h per day for one or two days resulted in a dose-related decrease in the ciliary activity of the tracheal epithelium and in mucosal degeneration (Adalis *et al.*, 1978).

A single intramuscular injection of nickel chloride (18.3 mg/kg bw) to male CBA/J mice caused significant involution of the thymus within two days. Significant reduction in the mitogen-stimulated responses of B and T lymphocytes *in vitro* as well as significant suppression of the primary antibody response (T-cell-dependent) to sheep red blood cells were observed after the treatment. Natural killer cell activity was also suppressed. The immunosuppressive effects of nickel chloride lasted for a few days. The activity of peritoneal macrophages was not affected (Smialowicz *et al.*, 1984, 1985).

Following a single intramuscular injection of 10-20 mg/kg bw nickel chloride into Fischer 344 rats, the activity of natural killer cells was transiently suppressed for three days. In contrast to mice, rats showed no significant difference in the lymphoproliferative responses of splenocytes to B and T mitogens from those of controls (Smialowicz *et al.*, 1987).

Intramuscular injection of nickel chloride (20 mg/kg bw Ni) to Fischer 344 rats 4 h before death inhibited thymidine uptake into kidney DNA (Hui & Sunderman, 1980). An immediate, significant decrease, followed by a transient sharp increase of thymidine incorporation into pulmonary DNA was observed in strain A mice following intraperitoneal administration of nickel acetate (Kasprzak & Poirier, 1985).

After 90 daily intraperitoneal injections of nickel sulfate (3 mg/kg bw Ni) to male albino rats, focal necrosis of the proximal convoluted tubules in the kidneys and marked cellularity around periportal areas and necrotic areas in the liver were observed. Bile-duct proliferation and Kupffer-cell hyperplasia were also evident, and degenerative changes were observed in a few seminiferous tubules and in the inner wall of the myocardium (Mathur *et al.*, 1977a).

Subcutaneous injection of up to 0.75 mmol [98 mg]/kg bw nickel chloride to male Fischer 344 rats increased lipid peroxidation in the liver, kidney and lung in a dose-related manner (Sunderman *et al.*, 1985b).

Renal, hepatic, pulmonary and brain haem oxygenase activity was induced after subcutaneous injection of 15 mg/kg bw nickel chloride to male Fischer 344 rats. Induction of haem oxygenase was also observed in the kidneys of male BL6 mice, male Syrian golden hamsters and male guinea-pigs killed 17 h after subcutaneous injection of 0.25 mmol [32 mg]/kg bw nickel chloride (Sunderman *et al.*, 1983a).

The skin of male albino rats was painted once daily for up to 30 days with 0.25 ml nickel sulfate hexahydrate solution in normal saline (40, 60 and 100 mg/kg bw Ni). The 30-day painting caused atrophy in some areas and acanthosis in other areas of the epidermis, disorder in the arrangement of epidermal cells and hyperkeratinization. Liver damage, including focal necrosis, was seen in histological studies (Mathur *et al.*, 1977b).

The toxicity of nickel sulfate and nickel chloride to alveolar macrophages from beagle dogs and Fischer 344 rats *in vitro* was intermediate to that of nickel subsulfide and nickel monoxide (median lethal concentrations, 0.30-0.36 μmol [17.7-21.2 μg] and 3.1-3.6 μmol [183-212 μg]/ml Ni for dog and rat macrophages, respectively) (Benson *et al.*, 1986a). Macrophages lavaged from rabbit lungs and incubated for two days with 3-24 μg/ml Ni as nickel chloride showed a decrease of up to 50% in lysozyme activity with increasing concentrations of nickel (Lundborg *et al.*, 1987).

Although nickel salts inhibit the proliferation of normal mammalian cells in culture, nickel sulfate hexahydrate increased proliferation of some lymphoblastoid cell lines carrying the Epstein-Barr virus (Wu *et al.*, 1986).

Exposure of Syrian hamster embryo cells to nickel chloride or nickel sulfate at a concentration of 10 μmol [600 μg]/l Ni or more enhanced nucleoside excretion (Uziel *et al.*, 1986).

Nickel chloride inhibited the transcription of calf thymus DNA and phage T4 DNA with *Escherichia coli* RNA polymerase in a concentration-dependent manner (0.01-10 mM [0.6-600 mg] Ni). It also stimulated RNA chain initiation at very low concentrations (maximal at 0.6 mg), followed by a progressive decrease in initiation at concentrations that significantly inhibited overall transcription (Niyogi *et al.*, 1981).

(v) *Other nickel compounds*

Animals exposed to nickel carbonyl by inhalation developed pulmonary oedema within 1 h. LC_{50} values (30-min exposure) reported include 67 mg/m^3 for mice, 240 mg/m^3 for rats and 190 mg/m^3 for cats (National Research Council, 1975). Even after administration by other routes, the lung is the main target organ (Hackett & Sunderman, 1969); the LD_{50} for rats was 65 mg/kg, 61 mg/kg and 38 mg/kg after intravenous, subcutaneous and intraperitoneal administration, respectively (National Research Council, 1975).

Male Wistar rats exposed by inhalation to 0.03-0.06 mg/l nickel carbonyl for 90 min three times a week for 52 weeks developed extensive inflammatory lesions in the lung, contiguous pericarditis and suppurative lesions of the thoracic walls. Squamous-cell metaplasia was present in bronchiectatic walls of several rats (Sunderman *et al.*, 1957).

Exposure of female Fischer 344 rats by inhalation to 1.2-6.4 μmol [0.2-1.1 mg]/l nickel carbonyl for 15 min caused acute hyperglycaemia (Horak *et al.*, 1978). Urinary excretion of proteins and amino acids indicated nephrotoxicity (Horak & Sunderman, 1980).

After exposure of rats to 0.6 mg/l nickel carbonyl by inhalation, RNA derived from the lung showed alterations in the phase transition curve, indicating disruption of hydrogen bonds (Sunderman, 1963). Nickel carbonyl administered

intravenously at an LD_{50} dose of 20 mg/kg bw nickel to Sprague-Dawley rats inhibited cortisone-induced hepatic tryptophan pyrrolase (Sunderman, 1967), orotic acid incorporation into liver RNA *in vivo* and *in vitro* (Beach & Sunderman, 1969, 1970) and incorporation of leucine into liver and lung protein (Witschi, 1972). Intravenous administration of nickel carbonyl to Fischer 344 rats (20 mg/kg bw nickel) caused a significant decrease in thymidine incorporation into liver and kidney DNA 4 h later (Hui & Sunderman, 1980).

The toxicity of 'nickel carbonate' to human embryo pulmonary epithelium L132 cells in culture did not differ significantly from that of nickel chloride at the same 25-150 μM concentration range applied (Hildebrand *et al.*, 1986). [The Working Group noted that the compound tested was most probably basic nickel carbonate.]

A highly significant correlation was found between carcinogenic potential and the incidence of erythrocytosis for various water-insoluble nickel compounds, including nickel selenide, nickel subselenide, nickel telluride, nickel sulfarsenide, nickel arsenide, nickel arsenide tetragonal, nickel arsenide hexagonal, nickel antimonide, nickel ferrosulfide matte, a ferronickel alloy ($NiFe_{1-6}$) and nickel titanate (Sunderman *et al.*, 1984b).

Dusts of nickel-converter mattes (58% nickel sulfide, 11% metallic nickel, 2% nickel monoxide, 1% copper, 0.5% cobalt, 0.2% soluble nickel salts), a nickel concentrate (67% total nickel, 57% nickel sulfide) and two nickel-copper mattes (27-33% nickel sulfides, ~3% metallic nickel, 23-36% copper) were administered to white rats and mice by inhalation or by intragastric, intratracheal or intraperitoneal routes and onto the skin. The intratracheal LD_{50} was 200-210 mg/kg bw for the mattes and 220 mg/kg for the nickel concentrate. The intraperitoneal LD_{50} varied from 940 mg/kg bw for the nickel concentrate to 1000 mg/kg bw for the nickel-copper mattes and 1100 mg/kg bw for the nickel matte. Mice and rats were almost equally sensitive. Chronic exposure of rats and mice by inhalation to the same dusts caused bronchitis, perivasculitis, bronchopneumonia and fibrosis. Haemorrhagic foci and atrophy were observed in the kidneys (Saknyn *et al.*, 1976).

(*e*) *Effects on reproduction and prenatal toxicity*

The embryotoxicity and genotoxicity of nickel, both directly to the mammalian embryo and indirectly through maternal injury, have been reviewed (Léonard & Jacquet, 1984).

(i) *Metallic nickel and nickel alloys*

Treatment of chick embryo myoblasts with 20-40 μg nickel powder per litre of culture fluid prevented normal differentiation of cells, with only a few mitoses seen after five days' incubation. Reduction of cell division was coupled with cell

degeneration, resulting in small colony size. At concentrations of 80 μg/l nickel, extensive degeneration of the cultures and complete suppression of mitosis occurred within five days (Daniel *et al.*, 1974).

(ii) *Nickel sulfides*

Nickel subsulfide (80 mg/kg bw Ni) administered intramuscularly to Fischer rats on day 6 of gestation reduced the mean number of live pups per dam. No anomaly was found, and no evidence of maternal toxicity was reported (Sunderman *et al.*, 1978a). In another study, intrarenal injection of nickel subsulfide (30 mg/kg bw Ni) to female rats prior to breeding produced intense erythrocytosis in pregnant dams but not in the pups, which had reduced blood haematocrits at two weeks (Sunderman *et al.*, 1983b).

Both rats and mice administered 5 or 10 mg/m^3 nickel subsulfide aerosols by inhalation for 12 days developed degeneration of testicular germinal epithelium (Benson *et al.*, 1987).

(iii) *Nickel salts*

Studies on the teratogenic effects of nickel chloride in chick embryos have produced conflicting results, perhaps due to differences in dose and route of administration. Cardiac anomalies (Gilani, 1982), exencephaly and distorted skeletal development (Gilani & Marano, 1980) have been reported, whereas some authors found no nickel-induced anomaly (Ridgway & Karnofsky, 1952; Anwer & Mehrotra, 1986).

Embryo cultures from BALB/c mice were used to determine the mechanism of preimplantation loss of embryos derived from matings three and four weeks after treatment of males with 40 or 56 mg/kg bw nickel nitrate. Treated and control animals were allowed to mate with superovulated females and the number of cleaved eggs and the development of embryos to blastocysts and implantations were counted. Neither the fertilizing capacity of spermatozoa nor the development of cultured embryos was influenced by a dose of 40 mg/kg bw. A dose of 56 mg/kg bw significantly reduced the fertilization rate but did not affect the development of two-cell embryos. The results suggest that preimplantation loss after exposure to nickel is due to toxic effects on spermatids and spermatogonia rather than to zygotic death (Jacquet & Mayence, 1982).

Following daily intragastric administration of 25 mg/kg bw nickel sulfate to male white rats over a period of 120 days, severe lesions in germ-cell development in the testis were observed (Waltscheva *et al.*, 1972). Rats administered nickel sulfate by inhalation for 12 days developed testicular degeneration (Benson *et al.*, 1988b).

Groups of three to five male albino rats received subcutaneous injections of 0.04 mmol [6.2 mg]/kg bw nickel sulfate either as a single dose or as daily doses for

up to 30 days. Treatment interfered to some degree with spermatogenesis, but this was temporary, and the testes ultimately recovered (Hoey, 1966).

Preimplantation embryos from NMRI mice (two- and four- to eight-cell stages) were cultured with nickel chloride hexahydrate; 10 μM (2.5 mg) adversely affected the development of day 2 embryos (two-cell stage), whereas 300 μM (71.3 mg) were required to affect day 3 embryos (eight-cell stage) (Storeng & Jonsen, 1980). In order to compare the effects of nickel chloride hexahydrate on mouse embryos treated *in vivo* by intraperitoneal injection during the preimplantation period, a single injection of 20 mg/kg bw nickel chloride was given to groups of female mice on day 1, 2, 3, 4, 5 or 6 of gestation. On day 19 of gestation, the implantation frequency in females treated on day 1 was much lower than that of controls. The litters of the control group were larger, and significantly so, among mice treated on days 1, 3 and 5 of gestation; the body weight of fetuses was also decreased on day 19. Nickel chloride may thus adversely affect mouse embryos during the passage through the oviduct, with subsequent effects after implantation. Data on maternal effects were not presented (Storeng & Jonsen, 1981).

Long-Evans rats born in a laboratory especially designed to avoid environmental contamination from trace metals were administered nickel [salt unspecified] at 5 mg/l in the drinking-water in five pairs. About one-third of the offspring in the first generation were runts, and one maternal death occurred. In the second generation, there were 10% young deaths with only 5% runts and, in the third generation, 21% young deaths with 6% runts. Thus, the size of the litters decreased somewhat with each generation and, with some failures in breeding, the number of rats was reduced (Schroeder & Mitchener, 1971). A subsequent study, reported in an abstract, found similar effects on reproduction through two generations of rats following administration of 500 mg/l nickel chloride in drinking-water. There was no decrease in maternal weight gain or other maternal effect (Kimmel *et al.*, 1986).

Nickel chloride was administered in the drinking-water to female rats at a concentration of 0.1 or 0.01 mg/l Ni for seven months and then during pregnancy. Embryonic mortality was 57% among nine rats exposed to the higher concentration, compared to 34% among eight controls. At the lower concentration no such difference was observed (Nadeenko *et al.*, 1979).

Nickel chloride (1.2-6.9 mg/kg bw Ni) was administered intraperitoneally to pregnant ICR mice on single days between days 7-11 of gestation. Increased resorption, decreased fetal weight, delayed skeletal ossification and a high incidence of malformations were observed in a dose-related fashion on gestation day 18. The malformations consisted of acephaly, exencephaly, cerebral hernia, open eyelids, cleft palate, micromelia, ankylosis of the extremities, club foot and other skeletal

abnormalities. Five of 27, 6/25 and 7/24 dams receiving 4.6 mg/kg bw or more died within 72 h after injection on days 9, 10 and 11 (Lu *et al.*, 1979).

Fischer rats were administered nickel chloride (16 mg/kg bw Ni) intramuscularly on day 8 of gestation. The body weight of fetuses on day 20 of gestation and of weanlings four to eight weeks after birth were reduced. No congenital anomaly was found in fetuses from nickel-treated dams, or in rats that received ten intramuscular injections of 2 mg/kg bw Ni as nickel chloride twice daily from day 6 to day 10 of gestation (Sunderman *et al.*, 1978a).

Groups of pregnant Wistar rats were given nickel chloride (1, 2 or 4 mg/kg bw Ni) by intraperitoneal injection on days 8, 12 and 16 of pregnancy and were sacrificed on day 20. More malformations occurred when nickel was administered during organogenesis than after, and their occurrence was maximal at dose levels that were toxic to dams. The abnormalities included hydrocephalus, haemorrhage, hydronephrosis, skeletal retardation and one heart defect (Mas *et al.*, 1985).

(iv) *Other nickel compounds*

Nickel carbonyl (11 mg/kg bw Ni) was injected intravenously into pregnant Fischer rats on day 7 of gestation. On day 20, fetal mortality was increased, the body weight of live pups was decreased and there was a 16% incidence of fetal malformations, including anophthalmia, microphthalmia, cystic lungs and hydronephrosis. No information was given regarding maternal toxicity (Sunderman *et al.*, 1983b).

Fischer rats were exposed on day 7 or 8 of gestation by inhalation to nickel carbonyl at concentrations of 80, 160 or 360 mg/m^3 for 15 min. Ophthalmic anomalies (anophthalmia and microphthalmia) were observed in 86/511 fetuses from 62 pregnancies; they were most prevalent at the highest dose level and were not observed when the compound was given on day 9 of gestation (Sunderman *et al.*, 1979b). In another experiment, pregnant rats exposed to 60 or 120 mg/m^3 nickel carbonyl by inhalation for 15 min on day 8 of gestation also had a high incidence of ocular anomalies. Maternal toxicity was not reported (Sunderman *et al.*, 1978b).

Groups of pregnant hamsters were administered 60 mg/m^3 nickel carbonyl by inhalation for 15 min on days 4, 5, 6, 7 or 8 of gestation. Dams were sacrificed on day 15 and the fetuses examined for malformation. Exposure on days 4 and 5 of gestation resulted in malformations in about 5.5% of the progeny, which included cystic lung, exencephaly, fused rib, anophthalmia, cleft palate and haemorrhage into the serous cavities. Nine of 14 dams lived until day 16 of gestation. Haemorrhages were not observed in controls. Among the fetuses of dams exposed to nickel carbonyl on day 6 or 7 of gestation, one fetus had fused ribs and two had hydronephrosis. For pregnancies allowed to reach full-term, there was no significant difference on the day of delivery between pups from nickel carbonyl-exposed litters and controls. Neonatal mortality was increased (Sunderman *et al.*, 1980).

(f) *Genetic and related effects*

Many reviews of the genetic effects of nickel compounds have been published (Heck & Costa, 1982; Christie & Costa, 1984; Costa & Heck, 1984; Hansen & Stern, 1984; Reith & Brøgger, 1984; Costa & Heck, 1986; Fairhurst & Illing, 1987; Sunderman, 1989).

The genotoxic effects of different nickel compounds are divided into five categories: (i) those for metallic nickel; (ii) those for nickel oxides and hydroxides; (iii) those for crystalline nickel sulfide, crystalline nickel subsulfide and amorphous nickel sulfide; (iv) those for nickel chloride, nickel sulfate, nickel acetate and nickel nitrate; and (v) those for nickel carbonate, nickelocene, nickel potassium cyanide and nickel subselenide. The studies on these compounds are summarized in Appendix 1 to this volume.

(i) *Metallic nickel*

Nickel powder was reported not to induce chromosomal aberrations in cultured human peripheral lymphocytes [details not given] (Paton & Allison, 1972).

Nickel powder ground to a mean particle size of 4-5 μm at concentrations of 5, 10 and 20 μg/ml caused a dose-dependent increase in morphological transformation of Syrian hamster embryo cells (Costa *et al.*, 1981b). At 20 μg/ml, nickel powder produced a 3% incidence of transformation, while crystalline nickel subsulfide and crystalline nickel sulfide (at 10-20 μg/ml) produced a 10-13% incidence of transformation and 5 and 10 μg/ml of amorphous nickel sulfide induced none. Nickel powder inhibited progression through S phase in Chinese hamster CHO cells, as measured by flow cytometry (Costa *et al.*, 1982).

Hansen and Stern (1984) reported that nickel powder transformed BHK 21 cells [see General Remarks for concern about this assay]. Proliferation in soft agar was used as the endpoint. At equally toxic doses, they found that nickel powder and crystalline nickel subsulfide had similar transforming activities; the toxicity of 200 μg/ml nickel powder was equal to that of 10 μg/ml nickel subsulfide.

(ii) *Nickel oxides*

Nickel monoxide and nickel trioxide in distilled water gave negative results in the *Bacillus subtilis rec*$^+$/*rec*$^-$ assay for differential toxicity at concentrations ranging from 5 to 50 mM (Kanematsu *et al.*, 1980). [The Working Group noted that since particulate nickel compounds such as these are relatively insoluble and their entry into mammalian cells requires phagocytosis (Costa & Mollenhauer, 1980a,b,c), it is unlikely that they were able to enter the bacteria.]

Chromosomal aberrations were not induced in human peripheral lymphocytes by treatment *in vitro* with nickel monoxide [details not given] (Paton & Allison, 1972).

Nickel monoxide and nickel trioxide transformed Syrian hamster embryo cells at concentrations of 5-20 μg/ml. The activity of the trioxide was about twice that of the monoxide, similar to that of metallic nickel and about 20% that of crystalline nickel sulfide (Costa *et al.*, 1981a,b).

Nickel monoxide that was calcined at a low temperature had greater transforming activity in this system than nickel monoxide calcined at a high temperature at concentrations of 5 and 10 μg/ml and was equivalent to that of crystalline nickel sulfide. The cell-transforming activity of these nickel compounds was reported to correlate well with their ability to induce preneoplastic changes in rats (Sunderman *et al.*, 1987).

Syrian hamster BHK 21 cells were transformed by nickel monoxide and by a nickel oxide catalyst identified as $NiO_{1.4}(3H_2O)$. At equally toxic doses, nickel monoxide had the same transforming activity as did nickel subsulfide. [See General Remarks for concern about this assay.] The nickel oxide catalyst, $NiO_{1.4}$, had similar toxicity and transforming capacity as nickel subsulfide (Hansen & Stern, 1983, 1984).

The ability of 50 μM nickel monoxide to induce anchorage-independent growth in primary human diploid foreskin fibroblasts was similar to that of 10 μM nickel subsulfide or nickel acetate. The absolute numbers of anchorage-independent colonies induced at these doses were 26 with nickel monoxide, 67 with nickel subsulfide, 79 with nickel sulfide (10 μM) and about 42 with nickel acetate, compared with none in cultures of untreated cells. The frequency of anchorage-independent growth induced by nickel monoxide was about three-fold less than with nickel subsulfide, but was equivalent to that obtained with nickel acetate. The tranformed cells had 33- to 429-fold higher plating efficiency in agar than the parental cells; anchorage-independence was stable for eight passages only (Biedermann & Landolph (1987).

Nickel oxide inhibited progression through S phase in Chinese hamster CHO cells, as measured by flow cytometry (Costa *et al.*, 1982).

(iii) *Nickel sulfides (crystalline nickel sulfide, crystalline nickel subsulfide and amorphous nickel sulfide)*

Crystalline nickel sulfide and nickel subsulfide were actively phagocytized by cells at an early stage following their addition to tissue cultures. Phagocytosis was dependent upon the calcium concentration in the medium (Abbracchio *et al.*, 1982a) and particle size (particles >5-6 μm were much less actively taken up and much less toxic than smaller particles) (Costa & Mollenhauer, 1980a,b,c). Particles

are taken up in areas of active cell ruffling, internalized and moved about the cell in a saltatory motion; lysosomes repeatedly interact with the particles, which are contained in the perinuclear region and sometimes inside cytoplasmic vacuoles, where they slowly dissolve, releasing nickel ions (Evans *et al.*, 1982). Interaction between lysosomes and nickel sulfide particles may result in exposure of the particles to the acidic content of the lysosomes, and this interaction may accelerate intracellular dissolution of crystalline nickel sulfide to ionic nickel (Abbracchio *et al.*, 1982a). In contrast, amorphous nickel sulfide and nickel particles were not significantly taken up by cells *in vitro* (Costa *et al.*, 1981a). Crystalline nickel sulfide particles differ from amorphous particles in that they have a negative surface charge, as shown using Z-potential measurements and binding of the particles to filter-paper discs offering different charges (Abbracchio *et al.*, 1982b). Alteration of the positive charge of amorphous nickel sulfide particles by treatment with lithium aluminium hydride results in activation of phagocytosis (Abbracchio *et al.*, 1982b; Costa, 1983).

Crystalline nickel sulfide was actively phagocytized by the protozoan *Paramoecium tetraurelia* and induced lethal genetic damage in parent cells. The activity of nickel subsulfide was more consistent than that of nickel sulfide, but both compounds produced higher mutagenic activities than glass beads, used as a control. The concentrations used ranged from 0.5 to 54 μg/ml; both compounds showed greatest mutagenicity at 0.5 μg/ml, as higher levels were toxic (Smith-Sonneborn *et al.*, 1983).

Crystalline nickel subsulfide at 5, 10 and 50 μg/ml inhibited DNA synthesis in the rat liver epithelial cell line T51B (Swierenga & McLean, 1985). Nickel subsulfide inhibited progression through S phase in Chinese hamster CHO cells, as measured by flow cytometry (Costa *et al.*, 1982).

Crystalline nickel sulfide and nickel subsulfide were active in inducing DNA damage in cultured mammalian cells. Crystalline nickel sulfide induced DNA strand breaks in rat primary hepatocytes (Sina *et al.*, 1983) and, at 1-20 μg/ml, single-strand breaks in tritium-labelled DNA in cultured Chinese hamster ovary cells, as determined using alkaline sucrose gradients (Robison & Costa, 1982). Using the same technique, Robison *et al.* (1982) showed that crystalline nickel subsulfide also induced strand breaks, whereas amorphous nickel sulfide, which is not phagocytized by cells, did not. As observed with alkaline elution analysis, crystalline nickel sulfide induced two major types of lesion — single-strand breaks and DNA protein cross-links (Costa *et al.*, 1982; Patierno & Costa, 1985). Treatment of primary Syrian hamster embryo cells with crystalline nickel subsulfide at 10 μg/ml and Chinese hamster CHO cells with crystalline nickel sulfide at 1-5 μg/ml induced DNA repair, as determined by analysis with caesium chloride gradients. Amorphous nickel sulfide had no effect in either cell type (Robison *et al.*, 1983).

Crystalline nickel subsulfide and amorphous nickel sulfide induced a weak mutation response at the *hprt* (6-thioguanine and 8-azaguanine resistance) locus in Chinese hamster ovary cells (Costa *et al.*, 1980).

Mutation to 8-azaguanine resistance was induced in a cultured rat liver epithelial cell line T51B treated with particulate crystalline nickel subsulfide at concentrations ranging from 5 to 50 μg/ml. At noncytotoxic doses, the mutagenic activity was four-fold above background, and at cytotoxic doses it was 20-fold above background. The mutagenic activity of dissolved products of these particles (at 12.5-20 μg/ml) was about two-fold above background at noncytotoxic doses and 20-fold above background at cytotoxic doses. Neither dissolved nor particulate nickel subsulfide at 2-27 μg/ml induced unscheduled DNA synthesis in rat primary hepatocytes (Swierenga & McLean, 1985). Nickel subsulfide, however, was reported to inhibit unscheduled DNA synthesis induced in primary rat hepatocytes by methyl methane sulfonate [details not given] (Swierenga & McLean, 1985). Concentrations of 0.5-10 μM nickel subsulfide did not induce 8-azaguanine or 6-thioguanine resistance in primary human fibroblasts (Biedermann & Landolph, 1987).

Crystalline nickel sulfide (0.1-0.8 $\mu g/cm^2$) was mutagenic in monolayer cultures in Chinese hamster V79 cells in which the endogenous *hprt* gene had been inactivated by a mutation and a single copy of a bacterial *gpt* gene had been inserted (Christie *et al.*, 1990).

The frequency of sister chromatid exchange was increased in cultured human lymphocytes treated with nickel subsulfide at 1-10 μg/ml (Saxholm *et al.*, 1981).

Chromosomal aberrations were induced in cultured mouse mammary carcinoma Fm3A cells following treatment with $4\text{-}8 \times 10^{-4}$M crystalline nickel sulfide dissolved in medium and filtered. The early chromosomal aberrations consisted of gaps; following reincubation in control medium after treatment, gaps, breaks, exchanges and other types of aberration were observed (Nishimura & Umeda, 1979; Umeda & Nishimura, 1979). [The Working Group noted that the chemical form of nickel used in this study is not known.]

Treatment of Chinese hamster ovary cells with crystalline nickel sulfide at 5-20 μg/ml for 6-48 h produced a dose- and time-dependent increase in the frequency of chromosomal aberrations, which were selective for heterochromatin and included mostly gaps and breaks, with some exchanges and dicentrics (Sen & Costa, 1985). Crystalline nickel sulfide at 1-10 μg/ml also increased the frequency of sister chromatid exchange in a dose-dependent fashion, selectively in heterochromatic regions, in both Chinese hamster ovary cells (Sen & Costa, 1986b) and mouse C3H/10T½ cells (Sen *et al.*, 1987).

A dose-dependent increase in the frequency of morphological transformation was induced in primary Syrian hamster embryo cells by treatment with crystalline nickel subsulfide at 1-5 μg/ml for nine days (DiPaolo & Casto, 1979) and by either crystalline nickel sulfide or nickel subsulfide at 0.1-10 μg/ml for 48 h (Costa *et al.*, 1979; Costa, 1980; Costa & Mollenhauer, 1980a,b,c; Costa *et al.*, 1981a,b, 1982). At the same dose range, amorphous nickel sulfide had no effect. Clones derived from the transformed cells had greater plating efficiency, saturation densities and proliferation rates than normal cells; they also had more inducibility of ornithine decarboxylase, were able to proliferate in soft agar and were tumorigenic in nude mice.

C3H/10T½ cells were transformed at equal frequencies by crystalline nickel subsulfide at concentrations of 0.001, 0.01 and 0.1 μg/ml; at concentrations higher than 1 μg/ml, there was no transformation due to cell lysis or death. Transformed cells also showed long microvilli. They were not characterized for their ability to form tumours in nude mice or for anchorage-independent growth (Saxholm *et al.*, 1981). [The Working Group questioned the induction of transformation by concentrations of crystalline nickel subsulfide as low as 0.001 μg/ml.]

Crystalline nickel subsulfide induced transformed properties in rat liver epithelial T51B cells that were related to cytokeratin lesions. Solutions prepared as leachates of nickel subsulfide (containing about 300 μg/ml Ni) induced large juxtanuclear cytokeratin aggregates within 24 h of exposure, which persisted after removal of the compounds and were passed on to daughter cells. After long-term exposure to 2.5 μg/ml crystalline nickel subsulfide (dissolution products), these lesions were related to concomitant induction of differentiation and transformation markers, loss of density dependence, ability to grow in calcium-deficient medium and increased growth rates. Altered cells formed differentiated benign tumours in nude mice (Swierenga *et al.*, 1989).

Crystalline nickel subsulfide at 5-20 μg/ml induced transformation to anchorage-independence of Syrian hamster BHK 21 cells (Hansen & Stern, 1983). [See General Remarks for concern about this assay.]

Human skin fibroblasts transformed by crystalline nickel subsulfide to anchorage-independent growth had a much higher plating efficiency than normal cells. The phenotype was stable for eight passages (Biedermann & Landolph, 1987).

Crystalline nickel sulfide, but not amorphous nickel sulfide, at doses of 1–20 μg/ml, inhibited the polyriboinosinic-polyribocytidylic acid-stimulated production of α/β interferon in mouse embryo fibroblasts (Sonnenfeld *et al.*, 1983).

Heterochromatic abnormalities were seen in early-passage cultures of cells from crystalline nickel sulfide-induced, mouse rhabdomyosarcomas (Christie *et al.*, 1988).

(iv) *Nickel salts (nickel chloride, nickel sulfate, nickel nitrate and nickel acetate)*

Nickel acetate induced λ prophage in *Escherichia coli* $WP2_s$, with a maximal effect at 0.04 mM (Rossmann *et al.*, 1984). Nickel sulfate at 300 μg/ml did not induce forward mutations in T4 phage (Corbett *et al.*, 1970).

Nickel chloride at 1-10 mM decreased the fidelity of DNA polymerase using a poly (c) template (Sirover & Loeb, 1976, 1977). Nickel acetate inhibited DNA synthesis in mouse mammary carcinoma Fm3A cells (Nishimura & Umeda, 1979).

Nickel chloride at 200-1000 μM induced a genotoxic response in a differential killing assay using *E. coli* WP2 (wild-type) and the repair-deficient derivative WP67 (*uvrA*$^-$, *polA*$^-$) and CM871 (*uvrA*$^-$, *recA*$^-$, *lexA*$^-$) (Tweats *et al.*, 1981). De Flora *et al.* (1984) reported negative results with nickel chloride, nickel nitrate and nickel acetate using the same strains in a liquid micromethod test procedure, with and without an exogenous metabolic system.

Nickel chloride did not induce differential toxicity in *B. subtilis* H17 *rec*$^+$ (*arg*$^-$, *trp*$^-$) or M45 *rec*$^-$ (*arg*$^-$, *trp*$^-$) at 5-500 mM (Nishioka, 1975; Kanematsu *et al.*, 1980). No mutagenicity was induced by nickel chloride at 0.1-100 mM in *S. typhimurium* LT_2 or TA100 (Tso & Fung, 1981), by nickel chloride, nickel acetate or nickel nitrate in *S. typhimurium* TA1535, TA1537, TA1538, TA97, TA98 or TA100 (De Flora *et al.*, 1984) or by nickel chloride or nickel sulfate in *S. typhimurium* TA1535, TA1537, TA1538, TA98 or TA100, when trimethylphosphate was substituted for *ortho*-phosphate to allow nickel to be soluble in the media (Arlauskas *et al.*, 1985). Even when substantial quantities of nickel were demonstrated to enter the bacteria, there was still no mutagenic response in *S. typhimurium* strains TA1535, TA1538, TA1975 or TA1978 (0.5-2 mM) (Biggart & Costa, 1986).

Pikálek and Nečásek (1983), however, demonstrated mutagenic activity of nickel chloride at 0.5-10 μg/ml in homoserine-dependent *Corynebacterium* sp887, utilizing a fluctuation test. Dubins and LaVelle (1986) demonstrated co-mutagenesis of nickel chloride with alkylating agents in *S. typhimurium* strain TA100 and in *E. coli* strains WP2$^+$ and WP2 *uvrA*$^-$; Ogawa *et al.* (1987) demonstrated co-mutagenesis with 9-aminoacridine. Nickel acetate at up to 100 μM was not co-mutagenic with ultraviolet light in *E. coli* WP2 (Rossman & Molina, 1986). Soluble nickel salts have been shown to be negative in host-mediated assays, using *S. typhimurium* G46 in NMRI mice and *Serratia marcescens* A21 in mice, at concentrations of 50 mg/kg (Buselmaier *et al.*, 1972).

Nickel chloride at 3 and 10 mM for 24 h induced gene conversion in *Saccharomyces cerevisiae* D7 (Fukunaga *et al.*, 1982). It also induced petite mutations in 13 *S. cerevisiae* haploid strains (Egilsson *et al.*, 1979).

Negative results were obtained in the *Drosophila melanogaster* somatic eye colour (zeste mutation) test with nickel chloride at 0.21 mM (Rasmuson, 1985) and at 4.2 mM (Vogel, 1976) and with nickel nitrate at 0.14 mM (Rasmuson, 1985).

Nickel sulfate induced sex-linked recessive lethal mutations in *D. melanogaster* at concentrations of 200, 300 and 400 ppm and sex chromosomal loss at the highest concentration tested. The injection volume was not stated, but the LD_{50} was 400 ppm (Rodriguez-Arnaiz & Ramos, 1986). Nickel nitrate at 3.4-6.9 mM did not induce sex-linked recessive lethal mutations in *D. melanogaster* (Rasmuson, 1985).

Nickel chloride increased the frequency of strand breaks in Chinese hamster ovary cells at 1 and 10 μg/ml with 2-h exposure (Robison & Costa, 1982) and at 10-100 μM for 16 and 48 h, with a decrease in the average molecular weight of DNA from 7.2-5.7 $\times$ 10^{-7} Da (Robison *et al.*, 1982). Nickel chloride at 0.5-5 mM induced both single-strand breaks and DNA-protein cross-links in the same cell line. The extent of cross-linking was maximal during the late S phase of the cell cycle when heterochromatic DNA is replicated (Patierno & Costa, 1985; Patierno *et al.*, 1985).

Nickel chloride at 0.05 mM for 30 min did not induce DNA strand breaks in human lymphocytes as evaluated by alkaline unwinding (McLean *et al.*, 1982). [The Working Group noted that the exposure period was very short and the dose very low.] Nickel sulfate at 250 μg/ml did not induce DNA single-strand breaks in human fibroblasts (Ag 1522) (Fornace, 1982).

Nickel chloride at 0.1-1 mM induced DNA repair synthesis in Chinese hamster ovary and primary Syrian hamster embryo cells, which have a very high degree of density inhibition of growth and very little background replication synthesis (Robison *et al.*, 1983, 1984). It inhibited DNA synthesis in primary rat embryo cells at 1.0 μg/ml (Basrur & Gilman, 1967) and in T51B rat liver epithelial cells (Swierenga & McLean, 1985).

Exposure of two human cell lines, HeLa and diploid embryonic fibroblasts, and of Chinese hamster V79 cells and L-A mouse fibroblasts to nickel chloride *in vitro* resulted in a dose-dependent depression of proliferation and mitotic rate. The effects on viability were accompanied by a reduction in DNA, protein and, to a lesser degree, RNA synthesis. Cells in G1 and early S phases were most sensitive (Škreb & Fischer, 1984). Nickel chloride also selectively blocked cell cycle progression in the S phase in Chinese hamster ovary cells (Harnett *et al.*, 1982). Nickel chloride at 40-120 μM for one to several days of exposure prolonged S-phase in Chinese hamster ovary cells (Costa *et al.*, 1982).

Nickel chloride at 0.4 and 0.8 mM for 20 h induced 8-azaguanine-resistant mutations in Chinese hamster V79 cells, although 0.8 mM induced a very weak mutagenic response (Miyaki *et al.*, 1979). Nickel chloride at 0.5-2.0 mM induced a dose-related increase in the frequency of mutation to 6-thioguanine resistance in

Chinese hamster V79 cells. At 2 mM, cell survival was 50% and the mutant fraction was 8.6-fold above background (Hartwig & Beyersmann, 1989). Trifluorothymidine-resistant mutants were induced in L5178Y $tk^{+/-}$ mouse lymphoma cells following exposure to nickel chloride at 0.17-0.71 mM for 3 h; dose-dependent two- to five-fold increases in mutation frequency were seen, survival ranging from 5 to 33.5% (Amacher & Paillet, 1980).

Nickel sulfate at 0.1 mM induced a two-fold increase in the frequency of mutation to 6-thioguanine resistance over the background level in Chinese hamster V79 cells (G12) containing a transfected bacterial *gpt* gene (Christie *et al.*, 1990). No gene mutation to ouabain resistance was seen, however, in primary Syrian hamster embryo cells exposed to 5 μg/ml nickel sulfate (Rivedal & Sanner, 1980).

As assessed in a mutation assay for the synthesis of P-85$^{gag\text{-}mos}$ viral proteins, nickel chloride at concentrations of 20-160 μM induced expression of the v-*mos* gene in MuSVts 110-infected rat kidney cells (6m2 cell line) (Biggart & Murphy, 1988).

Nickel chloride at 0.01-0.05 mM increased the incidence of sister chromatid exchange in Chinese hamster ovary cells (Sen *et al.*, 1987). An increased frequency was also seen with nickel sulfate at 0.1 mM in the P33 $8D_1$ macrophage cell line (Andersen, 1983), at 0.13 mM in Chinese hamster Don cells (Ohno *et al.*, 1982), at 0.004-0.019 mM in Syrian hamster embryo cells (Larramendy *et al.*, 1981), at 0.75 μg/ml (0.003 mM) in Chinese hamster ovary cells (Deng & Ou, 1981) and at 0.01 mM in human lymphocytes (Andersen, 1983). Dose-dependent increases in the frequency of sister chromatid exchange were seen in human peripheral blood lymphocytes with nickel sulfate at 0.01 mM and 0.019 mM (Larramendy *et al.*, 1981), 0.0023-2.33 mM (Wulf, 1980) and 0.95-2.85 μM (Deng & Ou, 1981).

Nickel chloride induced chromosomal aberrations in Fm3A mouse mammary carcinoma cells (Nishimura & Umeda, 1979; Umeda & Nishimura, 1979). It also induced aberrations (primarily gaps, breaks and exchanges) in Chinese hamster ovary cells at 0.001-1 mM, preferentially in heterochromatic regions (Sen & Costa, 1985, 1986b; Sen *et al.*, 1987); and aberrations in Syrian hamster embryo cells at 0.019 mM (Larramendy *et al.*, 1981). Increased frequencies were also reported in Syrian hamster embryo cells (0.019 mM) and human peripheral blood lymphocytes (0.019 mM) exposed to nickel sulfate hexahydrate (Larramendy *et al.*, 1981) and in Fm3A mouse mammary carcinoma cells exposed to nickel acetate at 0.6 mM for 48 h (Umeda & Nishimura, 1979) or at 1 mM for 24 h (Nishimura & Umeda, 1979).

Nickel sulfate at 1.0 mM reduced average chromosomal length in human lymphocytes, indicating its ability to act as a powerful spindle inhibitor at concentrations just below lethal levels (Andersen, 1985).

Nickel sulfate hexahydrate and nickel chloride induced a concentration-dependent increase in morphological transformation of Syrian hamster embryo cells (Pienta *et al.*, 1977; DiPaolo & Casto, 1979 [2.5-10 μg/ml]; Zhang & Barrett, 1988). Nickel sulfate transformed these cells at 5 μg/ml (Rivedal & Sanner, 1980; Rivedal *et al.*, 1980; Rivedal & Sanner, 1981, 1983), and concentrations of 10–40 μg/ml (38-154 μM) nickel sulfate enhanced transformation of normal rat kidney cells infected with Molony murine sarcoma virus (Wilson & Khoobyarian, 1982).

Nickel acetate at 100-400 μg/ml transformed Syrian hamster BHK21 cells (Hansen & Stern, 1983) [See General Remarks for concern about this assay.]

Nickel acetate and nickel sulfate at 10 μM induced transformation to anchorage-dependent growth of primary human foreskin fibroblasts (Biedermann & Landolph, 1987).

Continuous exposure of cultures of normal human bronchial epithelial cells to nickel sulfate at 5-20 μg/ml reduced colony-forming efficiency by 30-80%. After 40 days of incubation, 12 cell lines were derived which exhibited accelerated growth, aberrant squamous differentiation and loss of the requirement for epidermal growth factor for clonal growth. Aneuploidy was induced and marker chromosomes were found. However, none of these transformed cultures was anchorage-independent or produced tumours upon injection into athymic nude mice (Lechner *et al.*, 1984). Human fetal kidney cortex explants were exposed continuously to 5 μg/ml nickel sulfate. After 70-100 days, immortalized cell lines were obtained, with decreased serum dependence, increased plating efficiency, higher saturation density and ability to grow in soft agar. However, they were not tumorigenic (Tveito *et al.*, 1989).

Nickel sulfate disrupted cell-to-cell communication in a dose-related manner in NIH3T3 cells from a base level of 98% at 0.5 mM to 2% at 5mM; cell viability was not affected by these concentrations (Miki *et al.*, 1987). [The Working Group noted that the method for determining cell viability was not described.]

Intraperitoneal injections of nickel sulfate at 15-30% of the LD_{50} to CBA mice *in vivo* suppressed DNA synthesis in hepatic epithelial cells and in the kidney (Amlacher & Rudolph, 1981). Nickel chloride given by intramuscular injection to rats at 20 mg/kg bw Ni inhibited DNA synthesis in the kidney (Hui & Sunderman, 1980).

Polychromatic erythrocytes were not induced in BALB/c mice after an intraperitoneal injection of 25 mg/kg bw nickel chloride or 56 mg/kg bw nickel nitrate (Deknudt & Léonard, 1982).

The frequency of chromosomal aberrations in bone-marrow cells and spermatogonia of male albino rats was not increased following intraperitoneal injections of 3 and 6 mg/kg bw nickel sulfate. Animals were sacrificed seven to 14 days after treatment (Mathur *et al.*, 1978).

Nickel chloride increased the frequency of chromosomal aberrations in bone-marrow cells of Chinese hamsters given intraperitoneal injections of concentrations that were 4-20% of the LD_{50} (Chorvatovičová, 1983) and of Swiss mice given intraperitoneal injections of 6, 12 or 24 mg/kg bw (Mohanty, 1987).

Dominant lethal mutations were not induced in BALB/c mice after an intraperitoneal injection of 12.5-100 mg/kg bw nickel chloride or 28-224 mg/kg bw nickel nitrate (Deknudt & Léonard, 1982).

(v) *Other nickel compounds*

DNA-protein cross-linking in the presence of the nickel[II]- and nickel[III]-tetraglycine complexes and molecular oxygen was observed *in vitro* in calf thymus nucleohistone. The same complexes were also able to cause random polymerization of histones *in vitro* (Kasprzak & Bare, 1989).

Haworth *et al.* (1983) reported no mutation in *S. typhimurium* TA100, TA1535, TA1537 or TA98 following exposure to nickelocene at doses up to 666 μg/plate.

Nickel potassium cyanide at concentrations of 0.2-1.6 mM for 48 h increased the frequency of chromosomal aberrations in Fm3A mouse mammary carcinoma cells (Nishimura & Umeda, 1979; Umeda & Nishimura, 1979).

Crystalline nickel subselenide at 1-5 μg/ml inhibited cell progression through S phase, as seen with flow cytometry (Costa *et al.*, 1982). Concentrations of 5-20 μg/ml crystalline nickel subselenide transformed primary Syrian hamster embryo cells (Costa *et al.*, 1981a,b; Costa & Mallenhauer, 1980c).

Intravenous administration of nickel carbonyl to rats at 20 mg/kg bw Ni inhibited DNA synthesis in liver and kidney (Hui & Sunderman, 1980).

DNA-protein cross-links and single-strand breaks, as detected by alkaline elution, were found in rat kidney nuclei 20 h after intraperitoneal injection of 'nickel carbonate' at 10-40 mg/kg bw (Ciccarelli *et al.*, 1981). After 3 and 20 h, single-strand breaks were detected in lung and kidney nuclei, and both DNA-protein and DNA interstrand cross-links were found in kidney nuclei. No DNA damage was observed in liver or thymus gland nuclei (Ciccarelli & Wetterhahn, 1982). [The Working Group noted that the compound tested was probably basic nickel carbonate.]

3.3 Other relevant data in humans

(*a*) *Absorption, distribution, excretion and metabolism*

Recent reviews include those of Raithel and Schaller (1981), Sunderman *et al.* (1986a), the US Environmental Protection Agency (1986), Grandjean *et al.* (1988), Sunderman (1988) and the World Health Organization (1990).

A positive relation exists between air levels of nickel and serum/plasma concentrations of nickel after occupational exposure to various forms of nickel (see also

Tables 11, 12, 13). A considerable scattering of results was apparent, and the correlation was poor; a better correlation may be achieved in individual studies of well-defined exposure groups (Grandjean *et al.*, 1988). Sparingly soluble compounds may be retained in the lungs for long periods of time. Thus, even three to four years after cessation of exposure, nickel concentrations in plasma and urine were elevated in retired nickel workers exposed to sparingly soluble compounds in the roasting/smelting department of a nickel refinery (Boysen *et al.*, 1984). Respiratory uptake of nickel in welders is described in the monograph on welding.

Provided that pulmonary exposure to nickel can be excluded, the approximate fraction of nickel absorbed by the intestinal tract can be estimated from oral intake and faecal and urinary nickel elimination (Horak & Sunderman, 1973). Cumulative urinary excretion in non-fasting volunteers given a single oral dose of 5.6 mg Ni as nickel sulfate hexahydrate indicated an intestinal absorption of 1-5% (Christensen & Lagesson, 1981; Sunderman, 1988). After ingestion of nickel sulfate during fasting, 4-20% of the dose was excreted in the urine within 24 h (Cronin *et al.*, 1980). Compartmental analysis of nickel levels in serum, urine and faeces in a study of intestinal absorption of nickel sulfate by human volunteers showed that an average of about 27% was absorbed when ingested as an aqueous solution after 12 h of fasting, while 0.7% was absorbed when the nickel was ingested with scrambled eggs (Sunderman *et al.*, 1989b). Ingestion of food items with a high natural nickel content resulted in a urinary excretion corresponding to about 1% of the amount ingested (Nielsen *et al.*, 1987). The bioavailability of nickel can be reduced by various dietary constituents and beverages. Drugs may influence intestinal nickel absorption. Ethylenediaminetetraacetic acid very efficiently prevented intestinal absorption of nickel (Solomons *et al.*, 1982); and, as reported in an abstract, disulfiram increased the intestinal absorption of nickel, probably by forming a lipophilic complex between its metabolite diethyldithiocarbamate and nickel (Hopfer *et al.*, 1984).

After intestinal absorption of nickel ingested as nickel sulfate hexahydrate in lactose by eight volunteers, most of the nickel present in blood was in serum; nickel concentrations in serum and blood showed a very high positive correlation ($r = 0.99$) (Christensen & Lagesson, 1981). In patients with chronic renal failure, a high nickel concentration was found in serum but no significant increase was observed in lymphocytes (Wills *et al.*, 1985). However, in nickel refinery workers, plasma nickel concentrations were lower than those in whole blood, and about 63% appeared to be contained in the buffy coat (Barton *et al.*, 1980).

As reported in an abstract, nickel levels in intercellular fluid were significantly lower in a group of nickel-allergic patients than in controls, possibly due to cell binding or uptake (Bonde *et al.*, 1987). This finding may be related to the observation that incubation with nickel subsulfide *in vitro* caused considerable binding of

nickel to the cell membrane of T-lymphocytes from nickel-sensitized patients but to very few cells from nonsensitized persons (Hildebrand *et al.*, 1987).

The lungs contain the highest concentration of nickel in humans with no known occupational nickel exposure; lower levels occur in the kidneys, liver and other tissues (Sumino *et al.*, 1975; Rezuke *et al.*, 1987). One study documented high levels in the thyroid and adrenals (Rezuke *et al.*, 1987) and another in bone (Sumino *et al.*, 1975). The pulmonary burden of nickel appears to increase with age (Kollmeier *et al.*, 1987), although this correlation was not confirmed in another study (Raithel *et al.*, 1988). The upper areas of the lungs and the right middle lobe contained higher nickel concentrations than the rest of the lung (Raithel *et al.*, 1988), and high concentrations were found in hilar lymph nodes (Rezuke *et al.*, 1987).

Lung tissue from three of four random cases of bronchial carcinoma from an area with particularly high local emissions of chromium and nickel contained increased concentrations of nickel and chromium (Kollmeier *et al.*, 1987), while no such tendency was seen in ten other cases with no known occupational exposure to nickel (Turhan *et al.*, 1985).

High nickel concentrations were found in biopsies of nasal mucosa from both active and retired workers from the Kristiansand, Norway, nickel refinery, particularly in workers from the roasting/smelting department. After retirement, increased nickel levels persisted for at least ten years, with slow release at a half-time of 3.5 years (Torjussen & Andersen, 1979). Biopsies from two nasal carcinomas in nickel refinery workers contained nickel concentrations similar to those seen in biopsies from workers without cancer (Torjussen *et al.*, 1978). Lung tissue obtained at autopsy of workers from the roasting and smelting department of the Norwegian nickel refinery contained higher nickel concentrations (geometric mean, 148 μg/g dry weight; n = 15) than tissue from workers from the electrolysis department (geometric mean, 16 μg/g; n = 24); nickel concentrations in lung tissue were not higher in workers who had died from lung cancer than in workers who had died of other causes (Andersen & Svenes, 1989).

In cases of nickel carbonyl poisoning, the highest nickel concentrations have been recorded in the lungs, with lower levels in kidneys, liver and brain (National Research Council, 1975).

The half-time of nickel in serum was 11 h (one-compartment model during the first 32 h) in eight volunteers after ingestion of 5.6 mg nickel sulfate hexahydrate in lactose; serum nickel concentration and urinary nickel excretion showed a highly positive correlation ($r = 0.98$) (Christensen & Lagesson, 1981). Possibly due to delayed absorption of inhaled nickel, somewhat longer half-times were reported for nickel concentrations in plasma and urine (20-34 h and 17-39 h, respectively) in nickel platers (Tossavainen *et al.*, 1980), glass workers (30-50 h in urine) (Raithel *et*

al., 1982; Sunderman *et al.*, 1986a) and welders (53 h in urine) (Zober *et al.*, 1984). Ten subjects who had accidentally ingested soluble nickel compounds and were treated the following day with intravenous fluids to induce diuresis, showed an average elimination half-time of 27 h, while the half-time was twice as high in untreated subjects with lower serum nickel concentrations (Sunderman *et al.*, 1988b).

Urinary excretion of nickel is frequently used to survey workers exposed to inorganic nickel compounds (Aitio, 1984; Sunderman *et al.*, 1986a; Grandjean *et al.*, 1988). The best indicator of current exposure to soluble nickel compounds is a 24-h urine sample (Sunderman *et al.*, 1986a). In cases of nickel carbonyl intoxication, urinary nickel level is an important diagnostic and therapeutic guide (Sunderman & Sunderman, 1958; Adams, 1980), but its use in biological monitoring of exposure to nickel carbonyl has not been evaluated in detail.

Systemically absorbed nickel may be excreted through sweat (Christensen *et al.*, 1979). Faecal excretion includes non-absorbed nickel and nickel secreted into the gastrointestinal tract (World Health Organization, 1990). Saliva contains nickel concentrations similar to those seen in plasma (Catalanatto & Sunderman, 1977). Secretin-stimulated pancreatic juice was reported to contain an average of 1.09 nmol [64 μg]/ml nickel, corresponding to a total nickel secretion of about 1.64-2.18 μmol [96-128 μg] per day at a pancreatic secretion rate of 1.5-2 l/day (Ishihara *et al.*, 1987). Bile obtained at autopsy contained an average nickel concentration of 2.3 μg/l, suggesting daily biliary excretion of about 2-5 μg (Rezuke *et al.*, 1987). A biliary nickel concentration of 62 μg/g was recorded at autopsy of a small girl who had swallowed about 15 g nickel sulfate crystals (Daldrup *et al.*, 1983); since biliary excretion in this case would correspond to about 0.1% of the dose, it was considered that this route of excretion would be of minimal importance in acute intoxication (Rezuke *et al.*, 1987). Nickel-exposed battery production workers showed high faecal nickel excretion, probably owing to direct oral intake of nickel (e.g., *via* contamination of food from exposed surfaces); faecal nickel content (24 μg/g dry weight) was correlated with the amount present in air (18 μg/m^3) (Hassler *et al.*, 1983).

Nickel was found in cord blood from full-term infants at 3 μg/l (McNeely *et al.*, 1971). Tissue levels at 22-43 weeks of gestation were similar to those seen in adults (Casey & Robinson, 1978).

(b) Toxic effects

Nickel is an essential nutrient in several species, but no essential biochemical function has been established in humans. Recent reviews of nickel toxicity in humans include those of Raithel and Schaller (1981), the US Environmental Protection Agency (1986), Sunderman (1988) and the World Health Organization (1990).

Acute symptoms reported in 23 patients exposed to severe nickel contamination during haemodialysis included nausea, vomiting, weakness, headache and pal-

pitations; the symptoms disappeared rapidly upon cessation of dialysis (Webster *et al.*, 1980). Twenty workers who accidentally ingested water contaminated with nickel sulfate and chloride hexahydrates at doses estimated at 0.5-2.5 g Ni developed nausea, abdominal pain or discomfort, giddiness, lassitude, headache, diarrhoea, vomiting, coughing and shortness of breath; no related sequela was observed on physical examination, and all individuals were asymptomatic within three days (Sunderman *et al.*, 1988b). In a study of fasting human volunteers, one subject who ingested nickel sulfate (as 50 μg/kg bw Ni) in water developed a transient hemianopsia at the time of peak nickel concentration in serum (Sunderman *et al.*, 1989b). One fatal case of oral intoxication with nickel sulfate has been reported (Daldrup *et al.*, 1983).

Biochemical indications of nephrotoxicity, mainly with tubular dysfunction, have been observed in nickel electrolysis workers (Sunderman & Horak, 1981). Increased haemoglobin and reticulocyte counts were reported in ten subjects three to eight days after they had accidentally ingested 0.5-2.5 g Ni as nickel sulfate and chloride hexahydrates in contaminated drinking-water (Sunderman *et al.*, 1988b).

Nickel is a common skin allergen—in recent studies, the most frequent cause of allergic contact dermatitis in women and one of the most common causes in men; about 10-15% of the female population and 1-2% of males show allergic responses to nickel challenge (Peltonen, 1979; Menné *et al.*, 1982). Nickel ions are considered to be exclusively responsible for the immunological effects of nickel (Wahlberg, 1976). Sensitization appears to occur mainly in young persons, usually due to non-occupational skin exposures to nickel alloys (Menné *et al.*, 1982). Subsequent provocation of hand eczema may be caused by occupational exposures, especially to nickel-containing fluids and solutions (Rystedt & Fischer, 1983). Oral intake of low doses of nickel may provoke contact dermatitis in sensitized individuals (Veien *et al.*, 1985). Inflammatory reactions to nickel-containing prostheses and implants may occur in nickel-sensitive individuals (Lyell *et al.*, 1978).

Several cases of nickel-associated asthma have been described (Cirla *et al.*, 1985). Case reports suggest that inhalation of nickel dusts may result in chronic respiratory diseases (asthma, bronchitis and pneumoconiosis) (Sunderman, 1988). [The Working Group was unable to determine the causal significance of nickel in this regard.]

Nickel carbonyl is the most acutely toxic nickel compound. Symptoms following nickel carbonyl intoxication occur in two stages, separated by an almost symptom-free interval which usually lasts for several hours. Initially, the major symptoms are nausea, headache, vertigo, upper airway irritation and substernal pain, followed by interstitial pneumonitis with dyspnoea and cyanosis. Prostration, pulmonary oedema, kidney toxicity, adrenal insufficiency and death may occur in severe cases (Sunderman & Kincaid, 1954; Vuopala *et al.*, 1970; Sunderman, 1977).

Frequent clinical findings included fever with leukocytosis, electrocardiographic abnormalities suggestive of myocarditis and chest X-ray changes (Zhicheng, 1986). Hyperglycaemia has also been reported (Sunderman, 1977). Neurasthenic signs and weakness may persist in survivors for up to six months (Zhicheng, 1986).

(*c*) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(*d*) *Genetic and related effects*

Cytogenetic studies have been performed using peripheral blood lymphocytes from electroplating and nickel refining plant workers; they are summarized in Appendix 1 to this volume.

Waksvik and Boysen (1982) found elevated levels of chromosomal aberrations (mainly gaps; $p < 0.003$), but not of sister chromatid exchanges, in two groups of nickel refinery workers. One group of nine workers engaged in crushing/roasting/smelting processes and exposed mainly to nickel monoxide and nickel subsulfide for an average of 21.2 years (range, 3-33 years) at an air nickel content of 0.5 mg/m^3 (range, 0.1-1.0 mg/m^3) and with a mean plasma nickel level of 4.2 µg/l had 11.9% of metaphases with gaps. Another group of workers, engaged in electrolysis, who were exposed mainly to nickel chloride and nickel sulfate for an average of 25.5 years (range, 8-31 years) at an air nickel content of 0.2 mg/m^3 (range, 0.1-0.5 mg/m^3) and with a mean plasma level of 5.2 µg/l, had 18.3% of metaphases with gaps[1]. Mean control values of 3.7% of metaphases with gaps were seen in seven office workers in the same plant with plasma nickel levels of 1 µg/l, who were matched for age and sex. All subjects were nonsmokers and nonalcohol consumers, were free from overt viral disease, were not known to have cancer and had not received therapeutic radiation; none was a regular drug user and the groups were uniform as to previous exposure to diagnostic X-rays.

Waksvik *et al.* (1984) investigated nine ex-workers from the same plant who had been retired for an average of eight years who had had similar types of exposure to more than 1 mg/m^3 atmospheric nickel for 25 years or more; they were selected from among a group of workers known to have nasal dysplasia and who still had plasma nickel levels of 2 µg/l plasma. These retired workers showed some retention of gaps ($p < 0.05$) and an increased frequency of chromatid breaks to 4.1% of metaphases *versus* 0.5% ($p < 0.001$) in 11 unexposed retired workers controlled for age, life style and medication status. All subjects were of similar socioeconomic status and had

[1]The exposures of these workers were clarified in an erratum to the original article, published subsequently (*Mutat. Res.*, *104*, 395 (1982)).

not had X-rays or overt viral disease recently; none smoked or drank alcohol. Four exposed and nine unexposed subjects were on medication but not with drugs known to influence chromosomal parameters.

Deng *et al.* (1983, 1988) studied the frequencies of sister chromatid exchange and chromosomal aberrations in lymphocytes from seven electroplating workers exposed to nickel. Air nickel concentrations were 0.0053-0.094 mg/m^3 (mean, 0.024 mg/m^3). Control subjects were ten administrative workers from the same plant matched for age and sex; the groups were uniform as to socioeconomic status, and none of the subjects smoked or used alcohol, had overt viral disease, had recently been exposed to X-rays or was taking medication known to have chromosomal effects. The exposed workers had an increased frequency of sister chromatid exchange (7.50 $\pm$ 2.19 (SEM) *versus* 6.06 $\pm$ 2.30 (SEM); $p < 0.05$). [The Working Group noted that this is a small difference between groups.] The frequency of chromosomal aberrations (gaps, breaks and fragments) was increased from 0.8% of metaphases in controls to 4.3% in nickel platers.

The frequencies of sister chromatid exchange and chromosomal aberrations were studied in workers in a nickel carbonyl production plant. The subjects were divided into four groups: exposed, exposed smokers, controls and control smokers. Controls were ex-employees. None of the subjects had a history of serious illness; none was receiving irradiation or was infected by viruses at the time of blood sampling. No significant difference in the frequency of chromosomal breaks or gaps was observed between the different groups, and there was no statistically significant difference in the frequency of sister chromatid exchange between unexposed and nickel-exposed workers (Decheng *et al.*, 1987). [The Working Group noted that several discrepancies in the description of this study make it difficult to evaluate.]

Studies of mutagenicity and chromosomal effects in humans are summarized in Table 25.

3.4 Epidemiological studies of carcinogenicity to humans

(*a*) *Introduction*

The report of the International Committee on Nickel Carcinogenesis in Man (ICNCM) (1990) presents updated results on nine cohort studies and one case-control study of nickel workers, one of which was previously unpublished. The industries include mining, smelting, refining and high-nickel alloy manufacture and one industry in which pure nickel powder was used. The report adds to or supersedes previous publications on most of these cohorts, as various new analyses are included, some cohorts have been enlarged, and follow-up has been extended. Nickel species were divided into four categories: metallic nickel, oxidic nickel, soluble nickel and sulfidic nickel (including nickel subsulfide). Soluble nickel was defined as

Table 25. Cytogenetic studies of people exposed occupationally to nickel and nickel compounds

Occupational exposure	Reported principal components	Mean reported dose (range)	Sister chromatid exchange	Chromosomal aberrations	Reference
Crushing, roasting, smelting	Nickel monoxide, nickel subsulfide	Air: 0.5 (0.1–1.0) mg/m³ Exposure: 21.2 (3–33) years	None	Only gaps	Waksvik & Boysen (1982)
Electrolysis	Nickel chloride, nickel sulfate	Air: 0.2 (0.1–0.5) mg/m³ Exposure: 25.2 (8–31) years	None	Mainly gaps	Waksvik & Boysen (2982)
Crushing, roasting, smelting and/or electrolysis	Nickel monoxide, nickel subsulfide, nickel chloride, nickel sulfate	Air: 1 mg/m³ Exposure: > 25 years	None	Gaps and breaks in retired workers	Waksvik *et al.* (1984)
Nickel carbonyl production	Nickel carbonyl	Exposure: 7971 h	None	None	Decheng *et al.* (1987)
Electroplating	Nickel and chromium compounds	Air: 0.0053–0.094 mg/m³ Exposure: 2–27 years	Small increase	Mainly gaps, but also breaks and fragments	Deng *et al.* (1983, 1988)

consisting 'primarily of nickel sulfate and nickel chloride but may in some estimates include the less soluble nickel carbonate and nickel hydroxide'.

The historical estimates of exposure cited in the reviews of the following studies were not based on contemporary measurements. Furthermore, total airborne nickel was estimated first, and this estimate was then divided into estimates for four nickel species (metallic, oxidic, sulfidic and soluble), as defined in the report of the committee (ICNCM, 1990). The procedures for dividing the exposure estimates are described in section 2 of this monograph (pp. 297-298). Because of the inherent error and uncertainties in the procedures for estimating exposures, the estimated concentrations of nickel species in workplaces in the ICNCM analysis must be interpreted as broad ranges indicating only estimates of the order of magnitude of the actual exposures.

In order to facilitate the interpretation of the epidemiological findings on mortality from lung cancer and nasal cancer, selected estimates of exposure are presented in Tables 26, 27 and 28 (pp. 402-404) for some of the plants and subcohorts. The exposure estimates presented in the tables should be used only to make qualitative comparisons of exposure among departments within a plant and should not be used to make comparisons of exposure estimates among plants, for the reasons given above.

(*b*) *Nickel mining, smelting and refining*

(i) *INCO Ontario, Canada (mining, smelting and refining)*[1]

Follow-up of all sinter plant workers and of all men employed at the Ontario division of INCO for at least six months and who had worked (or been a pensioner) between 1 January 1950 and 31 December 1976 (total number of men, 54 509) was extended to the end of 1984 by record linkage to the Canadian Mortality Data Base (ICNCM, 1990). Sinter plant workers included men who had worked in two different sinter plants in the Sudbury area (the Coniston and Copper Cliff sinter plants) and in the leaching, calcining and sintering department at the Port Colborne nickel refinery. In the Coniston sinter plant, sulfidic nickel ore concentrates were partially oxidized at 600°C (Roberts *et al.*, 1984) on sinter machines to remove about one-third of the sulfur and to agglomerate the fine material for smelting in a blast furnace. In the Copper Cliff sinter plant, nickel subsulfide was oxidized to nickel oxide at very high temperatures (1650°C). The leaching, calcining and sintering

[1]There are some discrepancies between the figures cited here and those reported by Roberts *et al.* (1990a,b), but the differences are not substantial.

department produced black and green nickel oxides from nickel subsulfide by a series of leaching and calcining operations. The department also included a sinter plant like that at Copper Cliff. Employment records for men employed in the department did not allow them to be assigned to individual leaching, calcining or sintering operations. Mortality up to the end of 1976 in this cohort of about 55 000 men was described by Roberts *et al.* (1984); an earlier study of 495 men employed at the Copper Cliff sinter plant was reported by Chovil *et al.* (1981). The nickel species to which men were exposed in dusty sintering operations were primarily oxidic and sulfidic nickel, and possibly soluble nickel at lower levels (see Table 26). High concentrations of nickel compounds were estimated in the Copper Cliff sinter plant, which ranged from 25-60 mg/m^3 Ni as nickel oxide and 15-35 mg/m^3 Ni as nickel subsulfide, with up to 4 mg/m^3 Ni soluble nickel as anhydrous nickel sulfate between 1948 and 1954. Among the 3769 sinter plant workers, there were 148 lung cancer deaths (standardized mortality ratio (SMR), 261; 95% confidence interval (CI), 220-306) and 25 nasal cancer deaths (SMR, 5073; 95% CI, 3282-7489). Among the 50 977 nonsinter workers in the cohort, there were 547 lung cancer deaths (SMR, 110; 95% CI, 101-120) and six nasal cancer deaths (SMR, 142; 95% CI, 52-309). The only other site for which cancer mortality was significantly elevated was the buccal cavity and pharynx (12 deaths in sinter plant workers: SMR, 211; 95% CI, 109-369; 35 deaths in other workers: SMR, 71; 95% CI, 49-99). The sinter plant workers had little or no excess risk during the first 15 years after starting work (no nasal cancer death; five lung cancer deaths; SMR, 158 [95% CI, 51-370]), and their subsequent relative risk increased with increasing duration of employment. There were also statistically significant excesses of mortality from lung cancer in men employed for 25 or more years in the Sudbury area, both in mining (129 deaths; SMR, 134 [95% CI, 112-159]) and in copper refining (24 deaths; SMR, 207 [95% CI, 133-308]). In the electrolysis department of the Port Colborne plant, workers were estimated to be exposed to low concentrations of metallic, oxidic, sulfidic and soluble nickel. Seven nasal cancer deaths occurred (SMR, 5385; 95% CI, 2165-11 094) in men who had spent over 15 years in the electrolysis department at Port Colborne; all seven had spent some time in the leaching, sintering and calcining area at the Sudbury site, although two had spent only three and seven months, respectively. Lung cancer mortality among workers in the electrolysis department with no exposure in leaching, calcining and sintering, but with 15 or more years since first exposure, gave an SMR of 88 (19 deaths; 95% CI, 53-137). There was a marked difference in the ratio of lung to nasal cancer excess between the Copper Cliff sinter plant and the Port Colborne leaching, calcining and sintering plant: 7:1 at Copper Cliff (63 observed lung cancers, minus 20.5 expected, *versus* six nasal cancers) and only about 2:1 at Port Colborne (72 observed lung cancers, minus 30.0 expected, *versus* 19 nasal cancers).

(ii) *Falconbridge, Ontario, Canada (mining and smelting)*

A cohort of 11 594 men employed at Falconbridge, Ontario, between 1950 and 1976, with at least six months' service, was previously followed up to the end of 1976 (Shannon *et al.*, 1984a,b). Follow-up has now been extended to the end of 1985 by record linkage to the Canadian Mortality Data Base (ICNCM, 1990). Expected numbers were calculated from Ontario provincial death rates. One death was due to nasal cancer, compared with 0.77 expected. The only cause of death showing a statistically significant excess in the overall analysis was lung cancer (114 deaths; SMR, 135; 95% CI, 111-162). Subdivision of the total cohort by duration of exposure in different areas and latency revealed no SMR for lung cancer that differed significantly from this moderate overall excess, but the highest SMRs occurred in men who had spent more than five years in the mines (46 deaths; SMR, 158; 95% CI, 116-211) or in the smelter (15 deaths; SMR, 163; 95% CI, 91-269). Men who had worked in the smelter are reported to have had low levels of exposure to pentlandite and pyrrhotite, sulfidic nickel, oxidic nickel and some exposure to nickel sulfate. Estimated total exposures to nickel in all areas of the facility were below 1 mg/m^3 Ni (ICNCM, 1990).

(iii) *INCO, Clydach, South Wales, UK (refining)*

The excess of lung and nasal sinus cancer among workers in the INCO refinery in Clydach, South Wales, which opened in 1902, was recognized over 50 years ago (Bridge, 1933). The first formal analyses of cancer mortality were carried out by Hill in 1939 and published by Morgan (1958), who identified calcining, furnaces and copper sulfate extraction as the most hazardous processes. Subsequent reports indicated that the risk had been greatly reduced by 1925 or 1930 (Doll, 1958; Doll *et al.*, 1970, 1977; Cuckle *et al.*, 1980); trends in risk with age at first exposure, period of first exposure and latency were analysed (Doll *et al.*, 1970; Peto *et al.*, 1984; Kaldor *et al.*, 1986). The cohort of 845 men employed prior to 1945 studied by Doll *et al.* (1970) has now been extended to include 2521 men employed for at least five years between 1902 and 1969, and followed up to the end of 1984 (ICNCM, 1990). Among 1348 men first employed before 1930 there were 172 lung cancer deaths (SMR, 393; 95% CI, 336-456) and 74 nasal cancer deaths (SMR, 21 120; 95% CI, 16 584-26 514); the highest risks were associated with calcining, furnaces and copper sulfate production. The calcining and furnace areas had high estimated levels of oxidic, sulfidic and metallic nickel (see Table 27). Until the late 1930s, the oxidic nickel consisted of nickel-copper oxide. Men in the copper plant were exposed to very high concentrations of nickel-copper oxide; they were also exposed to soluble nickel: the extraction of copper from the calcine involved the handling of large volumes of solutions containing 60 g/l nickel as nickel sulfate. Until 1923, arsenic present in sulfuric acid is believed to have accumulated at significant levels in several process departments,

mainly as nickel arsenides. The only other significantly elevated risks were an excess of five lung cancer deaths (SMR, 333; 95% CI, 108-776) and four nasal cancer deaths (SMR, 36 363; 95% CI, 9891-93 089) in men employed before 1930 with less than one year in calcining, furnace or copper sulfate but over five years in hydrometallurgy, an area in which exposure to soluble nickel was similar to that in other high-risk areas and exposures to oxidic nickel were an order of magnitude lower than in other high-risk areas, with negligible exposure to sulfidic nickel (see Table 27); and in the small subgroup of nickel plant cleaners (12 lung cancer deaths; SMR, 784 [95% CI, 402-1361]), who were highly exposed to metallic nickel (5 mg/m^3 Ni), oxidic nickel (6 mg /m^3 Ni) and sulfidic nickel ($>$ 10 mg/m^3 Ni), with negligible exposure to soluble nickel (ICNCM, 1990). A notable anomaly in the data for the whole refinery was the marked reduction in nasal cancer but not lung cancer mortality, when comparing men first exposed before 1920 and those first exposed between 1920 and 1925 (Peto *et al.*, 1984). The risk, although greatly reduced, may not have been entirely eliminated by 1930, as there were 44 lung cancers (SMR, 125 [95% CI, 91-168]) and one nasal cancer (SMR, 526 [95% CI, 13-3028]) among the 1173 later employees.

(iv) *Falconbridge, Kristiansand, Norway (refining)*

The cohort of 3250 men reported by ICNCM (1990) is restricted to men first employed in 1946-69 with at least one year's service and followed until the end of 1984. For each work area, average concentrations for the four categories of nickel (sulfidic nickel, metallic nickel, oxidic nickel and soluble nickel) were estimated as four ranges for three periods (1946-67, 1968-77 and 1978-84). The four ranges and the arithmetic average computed for each range were: low (0.3 mg/m^3), medium (1.3 mg/m^3), high (5 mg/m^3) and very high (10 mg/m^3). There were 77 lung cancer deaths (SMR, 262; 95% CI, 207-327), three nasal cancer deaths (SMR, 453; 95% CI, 93-1324) and a further four incident cases of nasal cancer. Five of the nasal cancer cases had spent their entire employment in the roasting, smelting and calcining department, where oxidic nickel was estimated to have been the predominant exposure, with lesser amounts of sulfidic and metallic nickel. Before 1953, arsenic was present in the feed materials, and significant contamination with nickel arsenides is believed to have occurred at various steps of the process. The remaining two cases were in electrolysis workers who were exposed mainly to soluble nickel (nickel sulfate until 1953 and nickel sulfate and nickel chloride solutions thereafter) and nickel-copper oxides. No other type of cancer occurred significantly in excess. Among men first employed after 1955, there have been 13 lung cancer deaths (SMR, 173 [95% CI, 92-296]) and no nasal cancer (0.2 expected). Several comparisons were made assuming 15 years' latency. The highest risk for lung cancer was seen among a group of workers who had worked in the electrolysis deparment but never in roast-

ing and smelting (30 deaths; SMR, 385; 95% CI, 259-549). In the group of workers who had worked in roasting and smelting but never in the electrolysis department, 14 lung cancer deaths were seen (SMR, 225; 95% CI, 122-377) (see also Table 28). In those who had spent no time in either of these departments, the SMR was 187 (six cases [95% CI, 68-406]). Although exposure to soluble nickel in the roasting, calcining and smelting department was initially estimated to be negligible, it was noted that soluble nickel was certainly present in the Kristiansand roasting department in larger amounts than had been allowed for, and to some extent in all smelter and calcining plants (ICNCM, 1990).

The overlapping cohort reported by Pedersen *et al.* (1973) and Magnus *et al.* (1982) included 2247 men employed for at least three years from when the plant began operation in 1910. Results for cancers diagnosed up to 1979 were presented by Magnus *et al.* (1982). There were 82 lung cancers [standardized incidence ratio (SIR), 373; 95% CI, 296-463] and 21 nasal cancers (SIR, 2630 [95% CI, 1625-4013]). Of the nasal cancers, eight occurred in men involved in roasting-smelting, eight in electrolysis workers, two in workers in other specified processes and three in administration, service and unspecified workers. The incidence of no other type of cancer was significantly elevated overall, although there were four laryngeal cancers (SIR, 670) among roasting and smelting workers. An analysis of lung cancer incidence in relation to smoking suggested an additive rather than a synergistic effect. Adjustment for national trends in lung cancer rates, assuming an additive effect of nickel exposure, suggested little or no reduction in lung cancer risk between men first employed in 1930-39 and those first employed in 1950-59. This contrasts with the marked reduction in nasal cancer risk.

(v) *Hanna Mining and Nickel Smelting, Oregon, USA*

A total of 1510 men who had worked for at least six months between 1953, when the plant opened, and 1977 were followed up to the end of 1983 (ICNCM, 1990). Expected numbers of deaths were those for the state of Oregon. A statistically significant excess of lung cancer was observed among men with less than one year of exposure (seven deaths; SMR, 265 [95% CI, 107-546]) but not in men with longer exposure (20 deaths; SMR, 127 [95% CI, 77-196]) or in the subgroup who had worked in areas with potentially high exposures (smelting, 'skull plant', refining and ferrosilicon plant; seven deaths; SMR, 113; 95% CI, 45-233). There was no nasal cancer, and no excess of other cancers (21 deaths; SMR, 65 [95% CI, 41-100]). Average airborne concentrations were estimated to have been 1 mg/m^3 Ni or less, even in areas with potentially high exposure, and in most areas were below 0.1 mg/m^3 Ni. The principal nickel compounds to which workers were exposed were nickel-containing silicate ore and iron-nickel oxide, with very little soluble nickel and no sulfidic nickel.

(vi) *Societé Le Nickel, New Caledonia (mining and smelting)*

Approximately 25% of the adult male population of New Caledonia has worked in nickel mines (silicate-oxide nickel ores) or smelters. Since the local rates for cancer of the lung and upper respiratory tract are higher than those in neighbouring islands, a small hospital-based case-control study was conducted (Lessard *et al.*, 1978). Of the 68 cases identified in 1970-74, 29 cases and 22/109 controls had been exposed to nickel, giving an age- and smoking-adjusted relative risk (RR) of 3.0. [The Working Group noted that control subjects were selected from among patients seen in the laboratory of one hospital, while cases were identified through a variety of sources. Selection bias could have contributed to the apparent excess risk.]

Another study showed no difference in the incidence of lung cancer (RR, 0.9, not significant) or of upper respiratory tract cancer (RR, 1.4; not significant) between nickel workers and the general population. In a case-control study conducted among the nickel workers, no association was found between cancers at these sites and exposure to total dust, nickeliferous dust, raw ore or calcined ore (Goldberg *et al.*, 1987). Subsequent analyses (Goldberg *et al.*, 1990) provided little evidence that people with lung and upper respiratory tract cancer had had greater exposure to nickel than controls. Exposure was principally to silicate oxides, complex oxides, sulfides, metallic iron-nickel alloy and soluble nickel. The estimated total airborne nickel concentration in the facility was estimated to be low (<2 mg/m^3 Ni) (ICNCM, 1990).

(vii) *Other studies of mining, smelting and refining*

Several studies have been published in which the results were not described in sufficient detail for evaluation. Saknyn and Shabynina (1970, 1973) reported elevated lung cancer mortality among process workers in four nickel smelters in the USSR (SMRs, 200, 280, 380, 400 [no observed numbers given]). Electrolysis workers, exposed mainly to nickel sulfate and nickel chloride, were reported to be at particularly high risk for lung cancer (SMR, 820); excesses of stomach cancer and soft-tissue sarcoma were also observed. Tatarskaya (1965, 1967) reported an excess of nasal cancer among electrolysis workers in the USSR.

Olejár *et al.* (1982) reported a marginal excess of lung cancer (based on eight cases) among workers in a Czechoslovak refinery.

One nasal sinus cancer and one lung cancer occurred among 129 men at the Outokumpu Oy refinery in Finland, but expected numbers were not calculated. Workers were exposed primarily to soluble nickel; the highest measurement recorded was 1.1 mg/m^3 Ni (ICNCM, 1990).

Egedahl and Rice (1984) found no excess risk among workers in a refinery in Alberta, Canada, but there were only two cases of lung cancer in the cohort (SIR, 83 [95% CI, 10-301]).

(c) *Nickel alloy and stainless-steel production*

(i) *Huntington Alloys (INCO), W. Virginia (refining and manufacture of high-nickel alloys)*

A cohort of 3208 men with at least one year's service before 1947 was followed up to the end of 1977 (Enterline & Marsh, 1982) and then to the end of 1984 (ICNCM, 1990). Workers were exposed to metallic, oxidic, sulfidic and soluble nickel at low levels, except in the calcining department where high levels of sulfidic nickel (4000 mg/m^3 Ni) were present. Average airborne exposures were estimated to have been below 1 mg/m^3 Ni in all areas except calcining. On the basis of the ICNCM report (1990), there was no significant overall excess of lung cancer (91 deaths; SMR, 97 [95% CI, 80-121]). There was a nonsignificant excess among men first employed before 1947 (when calcining ceased) with 30 or more years' service (40 deaths; SMR, 124; 95% CI, 88-169). The group who had worked in calcining for five or more years was too small for useful analysis (two lung cancers; SMR, 100; 95% CI, 12-361). Four deaths from nasal cancer occurred in the whole cohort, all in persons employed before 1948; two were coded on death certificates as nasal cancer (expected, 0.9) and two were classified on the death certificates as bone cancer. Two had not worked in calcining and three had never been exposed to nickel sulfides; one had also worked as a heel finisher in a shoe factory. There was no excess mortality from nonrespiratory cancers.

(ii) *Henry Wiggin, UK (high-nickel alloy plant)*

Mortality up to 1978 in a cohort of 1925 men employed for at least five years in a plant that opened in 1953 was reported by Cox *et al.* (1981). Follow-up has now been extended to April 1985 for 1907 men (ICNCM, 1990). Average exposures from 1975 on rarely exceeded 1 mg/m^3 Ni in any area, with an overall average of the order of 0.5 mg/m^3 Ni. Measurements taken since 1975 were stated probably to be underestimates of the level of exposure to oxidic and metallic nickel of workers in earlier periods. Soluble nickel was reported to constitute 14-49% of total nickel in various departments (Cox *et al.*, 1981). Thirty deaths were due to lung cancer (SMR, 98; 95% CI, 57-121), including 13 deaths among men employed for ten years or more in areas where they were exposed to nickel (SMR, 91; 95% CI, 57-149). Subdivision by duration of exposure or latency produced no evidence of increased lung cancer risk, and there was no nasal cancer. An excess of soft-tissue sarcoma was found, based on two cases (SMR, 769; 95% CI, 92-2769) (ICNCM, 1990).

(iii) *Twelve high-nickel alloy plants in the USA*

Mortality up to the end of 1977 among 28 261 workers (90% male) employed for at least one year in 12 high-nickel alloy plants in the USA, and still working at some time between 1956 and 1960, was reported by Redmond (1984). There were 332 lung cancer deaths (SMR, 109 [95% CI, 98-122]) and two nasal sinus cancer deaths (SMR, 93 [95% CI, 12-358]). The excess of lung cancer was confined to men employed for five or more years in 'allocated services', most of whom were maintenance workers (197 deaths; SMR, 127 [95% CI, 110-146]). Excess mortality was observed from liver cancer (31 deaths; SMR, 182 [95% CI, 124-259]) in all men, and from cancer of the large intestine (SMR, 223 [95% CI, 122-375]) among non-white men. No data on exposure were available, but the authors noted that there may have been exposure to asbestos in these plants.

(iv) *Twenty-six nickel-chromium alloy foundries in the USA*

A proportionate mortality analysis of 851 deaths among men ever employed in 26 nickel-chromium alloy foundries in the USA in 1968-79 (Cornell & Landis, 1984) showed no statistically significant excess of lung cancer (60 deaths; proportionate mortality ratio (PMR, 105 [95% CI, 80-135]) or other cancers (103 deaths; PMR, 87 [95% CI, 71-106]) in comparison with US males. No death was due to nasal cancer.

Lung cancer mortality in a cohort of foundry workers was investigated by Fletcher and Ades (1984). The cohort consisted of men hired between 1946 and 1965 in nine steel foundries in the UK and employed for at least one year. The 10 250 members of the cohort were followed up until the end of 1978 and assigned to 25 occupational categories according to information from personnel officers. Lung cancer mortality for the subcohort of fettlers and grinders in the fettling shop was higher than expected on the basis of mortality rates for England and Wales (32 cases; SMR, 195; 95% CI, 134-276). [The Working Group noted that these workers may have been exposed to chromium- and nickel-containing dusts.]

(v) *Seven stainless-steel and low-nickel alloy production plants in the USA*

A proportionate mortality analysis of 3323 deaths among white males ever employed in areas with potential exposure to nickel in seven stainless-steel and low-nickel alloy production plants (Cornell, 1984) showed no excess of lung cancer (218 deaths; PMR, 97 [95% CI, 85-111]) or of other cancers (419 deaths; PMR 91 [95% CI, 83-100]). There was no death from nasal cancer.

(*d*) *Other industrial exposures to nickel*

(i) *Two nickel-cadmium battery factories in the UK*

Kipling and Waterhouse (1967) reported an excess of prostatic cancer based on four cases among 248 men exposed for one year or longer in a nickel-cadmium bat-

tery factory. The cohort was enlarged to include 3025 workers (85% men) employed for at least one month (Sorahan & Waterhouse, 1983, 1985), and the most recent report included deaths up to the end of 1984 (Sorahan, 1987). Exposure categories were defined on the basis of exposure to cadmium. The authors commented that almost all jobs with high exposure to cadmium also entailed high exposure to nickel hydroxide, and there was also possible exposure to welding fumes (Sorahan & Waterhouse, 1983). The excess of prostatic cancer cases was confined to highly exposed workers, among whom there were eight cases (SIR, 402 [95% CI, 174-792]); in the remainder of the cohort there were seven (SIR, 78 [95% CI, 31-160]) (Sorahan & Waterhouse, 1985). An excess of cancer of the lung was seen (110 deaths; SMR, 130 [95% CI, 107-157]), and this showed a significant association with duration in 'high exposure' jobs, particularly among men first employed before 1947 (Sorahan, 1987).

(ii) *A nickel-cadmium battery factory in Sweden*

A total of 525 male workers in a Swedish nickel-cadmium battery factory employed for at least one year were followed up to 1980 (Andersson *et al.*, 1984). Six deaths were due to lung cancer (SMR, 120 [95% CI, 44-261]), four to prostatic cancer (SMR, 129 [95% CI, 35-330]) and one to nasopharyngeal cancer (SMR, > 1000). Cadmium levels prior to 1950 were said to have been about 1 mg/m^3 in some areas; nickel levels were reported as 'about five times higher', although no actual measurement was reported.

(iii) *A nickel and chromium plating factory in the UK*

A total of 2689 workers (48% male) employed in a nickel-chromium plating factory in the UK were followed to the end of 1983 by Sorahan *et al.* (1987). There was excess mortality from lung cancer (72 deaths; SMR, 150 [95% CI, 117-189]) and nasal cancer (three deaths; SMR, 1000 [95% CI, 206-2922]), but this was confined to workers whose initial employment had been as chrome bath platers, and the lung cancer excess was significantly related to duration of chrome bath work. An earlier study of 508 men employed only as nickel platers in the factory (Burges, 1980) showed no excess for any cancer except that of the stomach (eight deaths; SMR, 267); among men with more than one year's employment, the SMR for stomach cancer was 476 (adjusted for social class and region; four deaths [95% CI, 130-1219]). The SMR for lung cancer was 122 [95% CI, 59-224].

(iv) *A die-casting and electroplating plant in the USA*

A proportionate mortality analysis of 238 deaths (79% male) in workers employed for at least ten years in a die-casting and electroplating plant in the USA was reported by Silverstein *et al.* (1981). There was excess mortality from lung cancer (28 deaths; PMR 191 [95% CI, 127-276]) among white men, but not for cancer at any other site. The PMRs for lung cancer by duration of employment were 165 (< 15

years) and 209 (≥15 years), and those by latency were 178 (<22.5 years) and 211 (≥ 22.5 years). The authors noted that the workers had been exposed to chromium[VI], polycyclic aromatic hydrocarbons and various compounds of nickel.

(v) *Oak Ridge gaseous diffusion plant, Tennessee, USA*

Fine pure nickel powder is used as barrier material in uranium enrichment by gaseous diffusion. A cohort of 814 white men employed at any time before 1954 in the production of this material was followed up from 1948 to 1972 by Godbold and Tompkins (1979). Exposure was thus entirely to metallic nickel. Follow-up was extended to the end of 1977 by Cragle *et al.* (1984), and mortality up to the end of 1982 was reported by ICNCM (1990). The median concentration of nickel was about 0.13 mg/m^3, but high concentrations occurred in some areas. About 300 of the 814 men had been employed for a total of less than two years. There was no excess of lung cancer, either overall (nine deaths; SMR, 54; 95% CI, 25-103) or among men employed for 15 years or longer (five deaths; SMR, 109 [95% CI, 35-254]), and mortality from other cancers was close to that expected (29 deaths; SMR, 96 [95% CI, 64-137]) for the whole cohort. No death from nasal cancer occurred, but only 0.22 were expected. [The Working Group noted that measurements made in 1948-63 (Godbold & Tompkins, 1979) suggest that the average exposure may have been to 0.5 mg/m^3 Ni.]

(vi) *Aircraft engine factory, Connecticut, USA*

Bernacki *et al.* (1978b) compared the employment histories of 42 men at an aircraft engine factory in the USA who had died of lung cancer with those of 84 age-matched men who had died of causes other than cancer. The proportion classified as nickel-exposed was identical (26%) among cases and controls. Atmospheric nickel concentrations in the past were believed to have been < 1 mg/m^3.

(*e*) *Other studies*

Several studies have been reported in which occupational histories of nasal cancer patients were sought by interview with patients or relatives, from medical or other records, or from death certificates. Acheson *et al.* (1981), in a study of 1602 cases diagnosed in England and Wales over a five-year period, found an excess (29 cases; SMR, 250 [95% CI, 167-359]) in furnace and foundry workers, which was partly (but not entirely) due to the inclusion of seven process workers from the INCO (Clydach) nickel refinery (see above). Hernberg *et al.* (1983) studied 287 cases diagnosed in Denmark, Finland or Sweden over a 3.5-year period. The association with exposure to nickel (12 cases, five matched controls among 167 matched case-control pairs who were interviewed; odds ratio, 2.4; 95% CI, 0.9-6.6) was not statistically significant. All except one of the nickel-exposed cases (a nickel refinery worker) had also been classified as having exposure to chromium (odds ratio, 2.7;

95% CI, 1.1-6.6), which was significantly associated with nasal cancer risk. Brinton *et al.* (1984) recorded exposure to nickel in only one (RR, 1.8; 95% CI, 0.1-27.6) of 160 cases and one of 290 controls in a hospital-based study between 1970 and 1980 in North Carolina and Virginia. Roush *et al.* (1980) examined exposure to nickel, cutting oils and wood dust in a case-control study based on all sinonasal cancer deaths in Connecticut in 1935-75. Job titles were obtained from deaths certificates and city directories and were classified according to estimated airborne exposures. Ten of 216 cases and 49 of 662 controls were classified as having been exposed to nickel (RR, 0.71; 95% CI, 0.4-1.5).

Gérin *et al.* (1984) reported significantly more frequent exposure to nickel among 246 Canadian lung cancer patients (29 exposed; odds ratio, 3.1; 95% CI, 1.9-5.0) than among patients with other cancers. All 29 cases had also been exposed to chromium, and 20 (69%) had been exposed to stainless-steel welding fumes. In a case-control study of 326 Danish laryngeal cancer patients, Olsen and Sabroe (1984) found a statistically significant association with exposure to nickel from alloys, battery chemicals and chemicals used in plastics production (RR, 1.7; 95% CI, 1.2-2.5; adjusted for age, tobacco and alcohol consumption and sex).