the plant, and 2 cases of skin cancer were reported in an 11-year period. Rockstroh noted that workers were exposed to nickel, arsenic, cobalt, copper, bismuth, and benzopyrene at the nickel smelting plant, although data were not presented. Because only 2 cases of skin cancer, the type of cancer usually associated with exposure to arsenic, were found, whereas 45 cases of lung cancer were diagnosed, Rockstroh concluded that arsenic was not the only cancer-causing agent to which workers at the nickel smelter were exposed.

Animal Toxicity

(a) Carcinogenicity

(1) Inhalation or Intratracheal Exposures

Kasprzak et al [77], in a study reported in 1973, administered 5 mg of nickel subsulfide suspended in 0.1 ml of 5% sodium carboxymethyl cellulose in a single intratracheal injection to each of 13 male Wistar rats weighing about 200 g. The mean particle size was 10 μm (range 1-30) and penetration into the small bronchioles and alveolar spaces was verified by microscopic examination of lungs of other rats similarly exposed. All the rats survived through the observation period of 15 months and were subsequently killed for examination. One liver tumor, without metastases, was seen. The lungs of nine of the rats, including the rat with the liver tumor, were unaltered, but the lungs of the other four showed peribronchial adenomatoid proliferation, and two of these had inflammatory reactions in the bronchial walls.

In another study with nickel subsulfide, Ottolenghi et al [78] reported the effects on Fischer rats of both sexes following its

inhalation. A group of 226 rats was exposed to respirable nickel subsulfide dust (95% of the particles were smaller than 1.5 μm) at an average nickel concentration of 0.97 mg/cu m (SD=0.18), and 241 controls were exposed to filtered air. The exposure period was 6 hours/day, 5 days/week, for 78-84 weeks. Hexachlorotetrafluorobutane, an agent used to induce pulmonary infarction, was also injected intravenously (iv) into one-half of the control and treated animals. The authors reported that this agent had no effect on the induction of tumors in these rats. Surviving animals were observed for 30 weeks before being killed. All animals were necropsied except 18 treated and 26 control animals, which were lost because of advanced autolysis or cannibalism. There was no difference in 1st-year mortality between the treated and control group; however, the mortality of the treated animals increased rapidly in the final 26 weeks of exposure, resulting in a significant difference ($P<0.01$) in survival by the end of the observation period. Fewer than 5% of the exposed rats survived for 108 weeks, whereas 31% of the controls survived. Nickel subsulfide-treated rats began to lose weight after 60 weeks of exposure. At the conclusion of the study, the differences in weight between control and treated rats averaged 65 g for females and 50 g for males. At necropsy, animals exposed to nickel subsulfide exhibited abscessed, consolidated, and spotty lungs. Typical hyperplasia in both the bronchial and bronchoalveolar segments of the lung was observed in 133 of the 208 nickel subsulfide-treated rats and in 46 of 215 controls. Hyperchromatism of the epithelial cells or proliferation of columnar and cuboidal cells were observed in 106 treated rats and in 28 control rats. Squamous metaplastic changes of the bronchial and bronchoalveolar portions of the lung were also

observed more frequently in nickel subsulfide-treated rats (18.3%) than in controls (4.6%). Benign epithelial tumors (8 bronchial and 7 alveolar adenomas) were found in 15 treated rats; the only tumor in this classification in the control group was described as an alveolar papilloma. Fourteen treated rats had malignant tumors, 10 of glandular type (adenocarcinomas), 3 of surface epithelial origin (squamous-cell carcinomas), and 1 of fibrous tissue origin (fibrosarcoma), but only one malignancy was found in a control animal. Ottolenghi et al [78] concluded that nickel subsulfide was a pulmonary carcinogen when inhaled by rats and hypothesized that its carcinogenicity was related to its low water solubility.

Farrell and Davis [79] reported in 1974 the results of a study designed to determine the effects of various particles, including nickel oxide, carbon, ferric oxide, aluminum oxide, and cobalt oxide, on diethylnitrosamine (DEN) carcinogenesis. Combinations of DEN and particles were given to 5 groups of 50 Syrian hamsters equally matched by sex. Four other groups received DEN alone and 6 additional groups served as controls. The DEN was administered subcutaneously and the particles by intratracheal instillation. DEN (a total of 6 mg) or saline was given once a week for 12 weeks; this was followed by 30 equal weekly administrations of a particulate substance for a total dose of 120 mg. All the animals were observed for up to 68 additional weeks. In the 200 animals which received DEN alone, there were three nasal tumors, one of which was malignant. In the 250 animals given both particles and DEN, there were 13 tumors of the nasal cavity, 9 of which were malignant. Other tumors of the respiratory tract did not differ among the groups. In the hamsters treated with nickel

oxide alone, there were no tumors in the respiratory tract and no excess of tumors at other sites. Animals given nickel oxide and DEN had four nasal tumors and no excess of other tumors compared to groups given DEN. Nickel oxide increased the number of nasal tumors produced by DEN, but not significantly more than the other particles used in the study.

Wehner et al [80], in 1975, described the results of a nickel oxide inhalation study. A group of 102 two-month-old male Syrian golden hamsters received lifespan exposures (up to 2 years) to a respirable aerosol (median diameter, 0.3 μm) of nickel oxide at a concentration of 53.2 mg/cu m (SD=11.1), for 7 hours/day, 5 days/week. Half of the animals were also exposed for 10 minutes three times daily to cigarette smoke. Fifty-one additional animals were exposed to cigarette smoke and sham dust; another group of 51 control hamsters was exposed to sham smoke and sham dust. The first evidence of a nickel oxide effect was the accumulation of nickel oxide particles on the alveolar septa. Accumulation was noted in macrophages aggregated near small bronchioles, lymph vessels, and blood vessels. Animals which died soon after the initiation of the exposures were examined microscopically; emphysema and particles of nickel oxide, often filling entire alveolar spaces, were noted, but little cellular response was seen. Animals which survived longer showed an increasing cellular response characterized by inflammation, macrophage proliferation, and bronchial and bronchiolar epithelial hyperplasia. Three malignant tumors, two osteosarcomas and a rhabdomyosarcoma of the thoracic skeletal muscle, were observed in nickel oxide-exposed animals. The control animals did not develop these tumors. Differences in survival or body weight related to nickel oxide exposure alone were not recorded. The authors [80]

concluded that, whereas lung lesions (massive pneumoconiosis) developed from chronic exposure to nickel oxide, "neither a significant carcinogenic effect of the nickel oxide nor a cocarcinogenic effect of cigarette smoke" was found.

Kim et al [81], in an unpublished inhalation study, exposed male Wistar rats to various combinations of nickel and iron dusts. The 287 rats were divided into three treatment groups (77, 76, and 67 animals) and one control group (67 animals). Group I was exposed to nickel powder at a concentration of 87.3 μg/cu ft (SD=8.06) (3.1 mg/cu m). Group II was exposed to a mixture of equal weights of nickel powder, "Dust C" (24.1% nickel sulfate, 68.7% nickel sulfide (Ni_2S_3), and 7.2% nickel oxide), hematite (Fe_2O_3), and pyrrhotite (FeS); the total nickel concentration was 59.5 μg/cu ft (SD=5.60) (2.1 mg/cu m), and the iron concentration averaged 53.2 μg/cu ft (SD=5.70) (1.9 mg/cu m). Group III was exposed to an iron mixture (iron, hematite, and pyrrhotite) at an iron concentration of 85.0 μg/cu ft (SD=6.50) (3.0 mg/cu m). Within each group, subgroups were exposed from 7 to 16 months over a 10-26 month period; identical exposure schedules were used for all three dust combinations. Particle sizes were predominantly (98%) less than 2 μm. All animals were exposed 6 hours/day, 5 days/week. In Group I, 3 of 60 examined rats had lung tumors (two carcinomas and one lymphosarcoma); in Group II, there was 1 squamous-cell carcinoma of the lung in the 61 rats that were examined; in Group III, 3 of 58 rats had lung tumors (2 carcinomas and 1 papillary adenocarcinoma), and, in the 55 control animals, 1 lung carcinoma was found. Twelve of 40 rats in Group I and 11 of 36 rats in Group II had granulomas in the lungs, compared to only 1 of 55 control rats and 3 of 58 animals in Group III.

The authors [81] concluded that under the conditions of the experiment, there was no evidence that lung cancer was the result of a direct carcinogenic action of the inhaled dust.

Hueper [82], in 1958, reported a study of the carcinogenic potential of elemental nickel. A total of 222 animals were exposed to 99% pure nickel, 4 μm or less in size, at a concentration averaging 15 mg/cu m for 6 hours/day, 4 or 5 days/week, for 24 months or until death. Guinea pigs (32 males, 10 females), Wistar rats (50 males, 50 females), Bethesda rats (60 females), and C57 black mice (20 females) were exposed. By the end of the 1st year, 45% of the guinea pigs, 64% of the Wistar rats, 52% of the Bethesda rats, and 85% of the mice had died. All of the exposed animals died by the end of the 2nd year. Microscopic examination of the guinea pigs showed that signs of lung irritation (edema, hemorrhage, hyperemia, and leukocytic infiltration) were common. In addition, areas in the bronchioles and alveoli contained an increase in the number and size of unusually shaped and hyperchromatic epithelial cells. These areas also showed accumulations of adenomatoid cell formations which extended into adjacent areas as exposure progressed. In six of the animals the lungs contained circumscribed areas sufficiently atypical to be described as microcarcinomas or minature adenocarcinomas. A multicentric anaplastic alveolar carcinoma was found in one guinea pig lung. The only metastatic lesion found in the guinea pigs was a tumor, suspected by the author to have originated in the lung, observed in a lymph node near the bladder; however, the primary tumor was never located. Five of nine control guinea pigs of similar age also showed small adenomatoid peribronchial cellular accumulations but not of the degree or frequency seen in the treated

animals. The author [82] stated that the frequency, extent, and number of adenomatoid lesions in the guinea pigs increased with the length of exposure. In rats, the lungs of 15 of 50 examined animals showed "rather numerous" adenomatoid lesions or accumulations. Chronic paranasal sinus inflammations accompanied by ulcers were also noted in a majority of the rats. The mice showed general hyperemia and hemorrhagic lesions, probably from irritation, but no mucosal or adenomatoid lesions such as were found in guinea pigs and rats. Although suggestive lesions were found in rats and guinea pigs, the data presented are not a clear indication of carcinogenicity attributable to elemental nickel.

Hueper and Payne [83] injected by thoracotomy 0.02 ml of a suspension containing finely powdered metallic nickel (2 g/10 ml) in a 10% gelatin solution. Twenty female and 14 male 3-month-old Bethesda Black rats were given nickel injections in the right lung. Control animals were not mentioned. Eight of the animals died within 72 hours after exposure. One year later the procedure was repeated on the 23 survivors, and all of the animals were dead 24 months after the initial exposure. Three malignant tumors were observed: a spindle-cell sarcoma of the right lung, probably related to treatment, and two squamous-cell carcinomas, one in the uterine endometrium and another in the skin of the cheek, possibly not related to treatment. Pigmented fibrous tissue, sometimes containing areas of bronchiolar adenomatosis, developed in many of the injected lungs. Since no controls animal were used, no comparison with expected results can be made.

Hueper and Payne [83] also tested in rats and hamsters the carcinogenic potential of powdered nickel, particle size 1-3 μm, inhaled

with sulfur dioxide and powdered limestone. The limestone was added to prevent the nickel particles from forming conglomerates and also to dilute the nickel content and decrease its toxicity. Chamber concentrations of nickel were not specified; the animals were exposed for over 6 hours/day to a mineral mixture (3-4 parts nickel to 1 part limestone for the hamsters and 1 part nickel to 1 part limestone for the rats) fed into the chamber at 50-65 g/day, along with sulfur dioxide at a concentration of 20-35 ppm. One hundred male hamsters and 120 rats (60 males, 60 females) were exposed, and all died in a 24-month period. Control animals were not mentioned. Cancers of the lung were not observed in either the rats or the hamsters; hamster lungs showed scarcely any effects attributable to exposure. Although many of the rats exhibited inflammatory "fibrosing" changes with bronchiectasis, squamous-cell metaplasia of the epithelial lining, and peribronchial adenomatosis, none of the lesions was definitely malignant.

(2) Oral Administration

In 1974, Schroeder et al [84] described an investigation in which 52 male and 52 female Long-Evans rats were exposed from weaning until death to an unspecified soluble nickel salt at a concentration of 5 ppm nickel in their drinking water. A similar group of rats served as controls. The drinking water for the treated and control rats consisted of deionized spring water with the following essential metals: zinc, 50 ppm; manganese, 10 ppm; copper, 5 ppm; chromium, 5 ppm; cobalt, 1 ppm; and molybdenum, 1 ppm. The authors [84] estimated that the diet of both treated and control rats contained 0.44 μg nickel/g of food. They stated that the average daily nickel consumption of adult rats was 2.6 μg of nickel for controls and 37.6 μg for treated animals. No difference in

survival between nickel-treated and control rats was noted. The experimental group developed three sarcomas, two carcinomas, and eight benign tumors. The controls had six sarcomas, three carcinomas, two lymphomas, and six benign tumors. A slight increase (13.3%) above controls in the incidence of focal myocardial fibrosis was observed in the rats that received nickel. Schroeder et al [84] concluded that nickel at 5 ppm in drinking water was nontoxic, nontumorigenic, and noncarcinogenic and adversely affected neither longevity nor growth in rats.

In another experiment, Schroeder et al [85] reported that, of 74 Swiss mice given nickel acetate over their lifespan at 5 ppm in drinking water, 10 developed tumors; tumors were also noted in 33 of 104 control mice. Because the diet in this study [85] was subsequently considered to be chromium deficient, Schroeder and Mitchener [86] repeated the experiment in 1975. In the latter study, they reported on the life-term effects of nickel on 108 male and female Swiss mice given drinking water containing 5 ppm of nickel as nickel acetate. Fourteen tumors were noted in 81 necropsied treated animals and 19 tumors were noted in 88 necropsied control animals.

The results of these three studies [84-86] suggest that nickel ions, at a dose of 5 ppm in the drinking water, do not cause increases in the incidence of tumors in mice and rats. No studies were found to evaluate the carcinogenic potential of nickel at higher levels.

(3) Intramuscular Injection and Implantation

Numerous investigators have reported injection- or implantation-site rhabdomyosarcomas and fibrosarcomas in laboratory animals treated with nickel or its compounds. Gilman and Ruckerbauer [87] observed

a 40-70% tumor incidence in rats and mice injected with dust taken from a flue in a Canadian nickel refinery. The major components of that dust were later tested individually by Gilman [88]. No injection-site tumors in rats treated with nickel sulfate were found, but tumor incidences of 23-35% in two strains of mice and 41% in rats injected with nickel oxide and 33-53% in two strains of mice and 80% in rats injected with nickel subsulfide were reported.

Nickel subsulfide has subsequently been found to be a very potent inducer of injection-site sarcomas [89-94]. Herchen and Gilman [89] reported that 32 days of exposure were required for a 250-mg nickel subsulfide disc, implanted in the thigh muscles, to induce tumors. Gilman and Herchen [90] also reported that physical shapes of nickel (powder, chips, disc, or diffusion chamber) affected the latency period but not the incidence of injection-site tumors. Sunderman et al [91] reported a dose-response relationship, ie, 4 of 15 rats developed tumors at a dose of 0.6 mg, 11 of 15 at 1.2 mg, 13 of 15 at 2.5 mg, and 15 of 15 at 5 mg.

Nickel powder has also been injected into laboratory animals [94-100]. Furst and Schlauder [95] reported that rats were much more sensitive to tumor induction by injection with nickel than were hamsters. Hueper [96] reported that mice did not develop tumors when administered nickel powder suspensions by intramuscular (im) or iv injection, but that intrafemoral injection of nickel powder induced injection-site tumors in 27 of 100 rats. In rabbits, one of six developed a tumor from intrafemoral injection, but no tumors were noted in 10 rabbits given six weekly iv injections of nickel. In another study, Hueper [97] observed 4 injection-site tumors in 25 rats given intrafemoral injections of nickel and none in

20 rats given intranasal injections of nickel.

Nickel acetate induced tumors in 3% of the rats given three 7-mg muscle implants [101] and in 22% of the rats injected with 35 mg [99]. No injection-site tumors were noted in animals injected with 5.6 or 22.4 mg of nickel monosulfide* [94]. One rat in each group of 35 animals given three 7-mg injections of nickelic oxide or anhydrous nickel acetate developed a tumor [101]. Ten injection-site tumors were observed in 35 rats over a period of 20 months after three 7-mg injections of nickel carbonate [101]. Nickel chloride, nickel sulfate, and nickel ammonium sulfate did not produce injection-site tumors when given in three 7-mg doses [101]. Nickel fluoride and nickel hydroxide have also been reported to produce injection-site tumors [102].

(4) Other Routes of Exposure

Jasmin and Riopelle [103] studied nickel subsulfide-induced renal carcinomas in young (120-140 g) female Sprague-Dawley rats. Nickel subsulfide (5 mg) in saline or in glycerin was injected into each pole of the right kidney; glycerin was also injected intrarenally into one control group, and nickel subsulfide (10 mg) was given iv to another. All animals were observed for 12 months and palpated regularly. No renal carcinomas were observed in 20 rats given iv nickel subsulfide or in the 16 glycerine-injected animals. In the intrarenally injected groups, nickel subsulfide in glycerine produced renal carcinomas in 7 of 16 examined animals, and nickel subsulfide in saline produced 11 carcinomas in 24 animals. The control groups showed no tumors remote from the injection sites; eight mesentery tumors, four lung tumors, and one liver tumor were noted as

extensions of primary tumors in the animals injected intrarenally with nickel subsulfide.

In a second experiment, Jasmin and Riopelle [103] studied the effect of other metals injected intrarenally. Glycerin (control), nickel subsulfide, nickel monosulfide, metallic nickel, cobalt sulfide, cobalt, chromium, cadmium, lead, or gold was injected into both poles of the right kidney of Sprague-Dawley rats (16-20 rats were autopsied for each metal). Doses of 5 mg of each metal in 0.05 ml of glycerin were used. No renal carcinomas developed in any rats except those injected with nickel subsulfide (7 of 16). Two rats in the nickel subsulfide group also developed mammary growths, and 1 of 20 rats injected with metallic nickel had a rhabdomyosarcoma at an unspecified site.

Stoner et al [104] investigated the production of lung adenomas in strain A mice given multiple intraperitoneal (ip) injections of 13 metallic compounds, one of which was nickel acetate. Three groups of 20 mice received nickel acetate 3 times weekly for 8 weeks in doses totaling 72, 180, and 360 mg/kg. Three control groups were used: one group was given the vehicle, another group was treated with a positive carcinogen (urethane), and a third group received no treatment. All mice were killed 30 weeks after the first injection, and the average number of lung tumors/mouse was determined. The average number of tumors/mouse was 0.42 in the vehicle control group, 21.6 in the urethane controls, and 0.28 in animals given no treatment. At a nickel acetate dose of 360 mg/kg, there were 1.26 tumors/mouse ($P<0.01$); at 180 mg/kg, 0.71 tumors/mouse; and at 72 mg/kg, 0.67 tumors/mouse. The increases in tumors/mouse at the 180 and 72 mg/kg doses were not statistically significant.

Furst et al [105] injected 5 mg of nickel powder intrapleurally into 10 Fischer rats. The procedure was repeated at monthly intervals for a total of five treatments. Control animals received saline injections. Two intrapleural tumors were noted in the nickel-treated animals; no tumors were found in the controls. The first nickel-induced tumor appeared in just over 100 days.

Hueper [97] reported the results of intrapleural injections of nickel powder. Five monthly injections of 50 mg of nickel in suspension were given to 25 rats. Four site tumors were noted 17 months after the beginning of the experiment. Hueper [96] later reported that no site tumors developed in 50 mice given single intrapleural injections. A dose of 0.02 ml of 0.6% suspension of powdered nickel in a 2.5% gelatin-saline solution was used.

Shafer [106] reported an attempt to induce tumors in the submaxillary salivary glands of 25 rats by implantation of a 25% nickel-lanolin paste. No tumors developed in the 66-week observation period.

(b) Mutagenic Effects

The mutagenic potential of inorganic nickel compounds has not been adequately studied. McCann et al [107] have commented that the Ames Salmonella-microsome test is not suitable for metals because of the large amounts of magnesium salts, citrate, and phosphate required in the minimal medium.

Buselmaier et al [108] tested the mutagenicity of 16 pesticides, including nickel chloride, in the host-mediated assay. NMRI-strain mice were injected ip with histidine-dependent strain G46 Salmonella typhimurium or leucine-dependent strain a21 Serratia marcescens. Immediately after

injection of one of the strains of bacteria, six mice received a subcutaneous nickel chloride injection of 50 mg/kg. Three hours later, the mice were killed; the bacteria were recovered and cultured for 4 days, and back-mutants were counted. In neither test was nickel chloride found to be mutagenic, nor was it mutagenic in the same bacterial strains on plate tests.

Green et al [109] described in 1976 the use of a simplified fluctuation test involving the measurement of reversion to tryptophan independence by tryptophan-dependent Escherichia coli, strain WP2. Nickel chloride at concentrations of 5, 10, and 25 μg/ml of medium did not increase reversion to tryptophan independence.

Demerec et al [110] reported in 1950 a study of mutation to streptomycin nondependence in streptomycin-dependent E. coli (B/r/Sd-4). The bacteria were washed to remove streptomycin, exposed to nickel sulfate or nickel nitrate for 1-24 hours (exact details not given), and plated on streptomycin-deficient medium. After 7 days, the number of mutant colonies was counted. Nickel sulfate and nickel nitrate were reported as "not mutagenic"; no supporting data were provided.

In 1970, Corbett et al [111] reported the effects of nickel sulfate on bacteriophage T4. T4-infected E. coli were treated with nickel sulfate (300 μg/ml) during phage replication. Mutations in the rII DNA segment were determined through the use of permissive (E. coli B) and nonpermissive (E. coli KB) hosts. Nickel sulfate was toxic but not mutagenic in this system.

Lindegren et al [112], in 1958, observed nickel-ion induction of respiratory deficiency in yeast cells. Respiration-sufficient yeasts, ie, those with red color and containing less than 1% respiration-deficient cells under normal conditons, were grown in media containing 0.013, 0.025, 0.038, 0.050, or 0.075% nickel chloride hexahydrate. Haploid (14061), diploid (F-2), and tetraploid (11294 x 11296) strains of yeast were used. Respiratory deficiency in all three strains, identified by the white color of the cells, was observed on the medium containing the 0.05% concentration (46-65% of the cells deficient). The 0.075% medium was lethal to all cells. The authors [112] stated that this deficiency may result either from destruction or inactivation of a cytoplasmic granule or from a gene mutation. The high frequency of deficiency and its relative independence of ploidy suggested to the authors [112] that the respiratory deficiency produced by nickel chloride was a cytotoxic effect rather than a gene mutation.

Nishioka [113] tested 0.05 ml of 0.05 M nickel chloride solution in the Rec assay with negative results. Although this assay does not measure mutagenic effects directly, it indicates qualitatively the ability of a chemical to induce DNA changes by measuring its inhibiting effect on growth of recombination-deficient Bacillus subtilis, strain M45, relative to the inhibition of growth in recombination-positive B. subtilis, strain H17.

In 1963, Komczynski et al [114] reported the effects of nickel chloride, nickel sulfate, and nickel nitrate on cell mitosis in the root of the broad bean (Vicia faba). Five concentrations (0.1, 0.01, 0.001, 0.0001, and 0.00001%) of each salt were tested. All of the nickel salts produced more abnormal cell divisions than were found in water-treated

controls. The nitrate salt was most effective, and the number of abnormal divisions increased slightly with the strength of the nitrate solution. Aberrations included abnormal arrangement of chromatin, grossly deformed cells, extra micronucleoli, and the appearance of small granulations of nuclear chromatin in the cytoplasm. The authors stated that these abnormalities are evidence of cell nuclei disturbances.

Von Rosen [115,116], in review papers, reported that nickel salts exhibited only weak genetic action on pea (Pisum abyssinicum) rootlets. Details of the studies were not given.

(c) Teratogenicity and Other Effects on Reproduction

In 1972, Ferm [117] cited previously unpublished data from his laboratory in a review of the teratogenic effects of metals on mammalian embryos. He presented data from the iv injection of nickel acetate into golden hamsters on the 8th day of pregnancy. Results are summarized in Table III-14 [117]. The author [117] reported that "a few general malformations in some of the surviving embryos" were noted, but details were not given. Lack of information on the malformations and on their frequency in various litters makes interpretation of or conclusions from this study uncertain.

TABLE III-14

EMBRYOCIDAL EFFECTS OF NICKEL ACETATE ON GOLDEN HAMSTERS

Amount Injected in mg/kg	Number of Pregnant Hamsters	Total Number of Embryos	Number of Embryos Resorbed	Total Number of Surviving Embryos	Number of Normal Embryos	Number of Abnormal Embryos
2	2	24	0	24	22	2
5	2	22	1	21	20	1
10	5	56	22	34	32	2
20	5	55	10	45	44	1
25	6	68	59	9	5	4
30	3	33	33	0	-	-

Adapted from Ferm [117]

Sunderman et al [118] recently reported in an abstract the fetal toxicity of nickel chloride administered im to Fischer rats on the 8th day of gestation. Groups of 12, 11, 12, and 13 rats were given nickel at 8, 12, 16, and 0 mg/kg. The dams were killed on the 20th day of gestation and live and dead fetuses were counted. The average number of live fetuses/dam was 9.7 in controls; 8.9 at the 8 mg dose; 7.7 at 12 mg ($P<0.001$); and 7.0 at 16 mg ($P<0.01$). The ratio of dead fetuses to the total number conceived was 2/128 in controls; 6/113 at 8 mg ($P<0.05$); 8/93 at 12 mg ($P<0.01$); and 19/103 at 16 mg ($P<0.001$). No fetal malformations were found at any treatment level. The authors concluded that im injections of nickel chloride caused fetal mortality at doses which did not cause maternal mortality.

The effect of hydrated nickel chloride on the developing chick embryo was described in 1952 by Ridgway and Karnofsky [119]. LD50 doses of nickel

chloride were injected into the yolk sac of 4-day embryos or into the yolk sac and onto the chorioallantoic membrane of 8-day embryos. The LD50 doses, determined in unreported trials, were 0.20 mg/egg for 4-day eggs, 2.38 mg/egg for 8-day yolk-sac-injected eggs, and 0.33 mg/egg for 8-day chorioallantoic-membrane-injected eggs. Although the toxicity varied with the age of the embryo and the route of exposure, the authors [119] reported that no developmental abnormalities were produced in chick embryos surviving the LD50 doses.

Timourian and Watchmaker [120] exposed sea urchin eggs to nickel chloride solutions and noted their subsequent development. Embryos were exposed at fertilization and followed through the developmental process. Nickel was incorporated into the fertilized egg more rapidly than certain other divalent ions. Early development, through the blastula stage, was not affected by nickel chloride concentrations up to 10 mM. However, gastrulation (normally occurring 18-20 hours after fertilization) was completely arrested in 10 mM solutions; gastrulation started in a few embryos, but was not completed in a 1 mM solution; and it only reached mid-development in a 0.1 mM solution. Eggs in 10 μM and 1 μM solutions completed gastrulation at the same time as controls. However, many of the embryos completing gastrulation were affected at later stages; at 10 μM, there were cell malformations, and at 1 μM, the prism stage was affected. In another test, embryos not exposed until the gastrula stage was reached were less affected than cells exposed at fertilization. Although nickel chloride did not grossly affect pregastrula development, the authors [120] concluded that, since it was absorbed in early stages, there were subtle effects on early morphologic development.

In 1971, Schroeder and Mitchener [121] described the effects of soluble nickel salts on reproduction in Long-Evans rats. Five pairs of weanlings were given nickel salts at 5 ppm in their drinking water. In previous experiments, Schroeder et al [84] had found that this concentration of soluble nickel salts had no effect on survival or growth of rats. In the present study [121], the litters which resulted from the subsequent mating of the treated rats formed the F1 generation. The F1 rats also received nickel in their drinking water and were mated to produce an F2 generation. The same procedure was repeated in the production of an F3 generation. Control animals received deionized water without nickel and were similarly raised through the F3 generation. Criteria used to estimate toxicity included intervals between litters, age when a pair produced its first litter, male-female ratio, number of runts (large head and small body), deaths, stillborn offspring, failure to breed, and congenital abnormalities. There was no effect on the interval between litters or the age at which the pair produced the first litter. In the treated F1 generation, 9.1% of the pups died and 30.6% were runts; in the treated F2 generation, 10.2% of the pups died and 5.1% were runts; and in the treated F3 generation, 21% of the pups died and 6.2% were runts. One dam from the F1 generation died; no other maternal deaths were reported. The average litter size in the nickel-treated rats decreased from 11.0 pups in the F1 generation to 8.1 in the F3 generation; the male-female ratio in these litters also decreased markedly, from 1.2 in the F1 to 0.44 in the F3 generation. In the controls there was one runt in the F2 generation and one pup died in the F3 generation; there were no changes in litter size or in the male-female ratio. The authors [121] concluded that nickel at 5 ppm

in the drinking water affected reproduction in rats.

Hoey [122], in 1966, reported the effects on rat testes of subcutaneous injections of nickel sulfate. Five albino rats were injected with nickel sulfate at 0.04 millimole/kg. Microscopic examination, 18 hours after a single injection, indicated that marked damage (central tubular shrinkage and hyperemia) had occurred in all testicular tissue except the interstitial tissue. Degeneration of the spermatozoa in the epididymis was noted. Four other animals received up to 30 daily injections of nickel sulfate at 0.04 millimole/kg, and one was killed on each of days 2, 10, 21, and 30. Although the interstitial tissue, the body of the epididymis, the Sertoli cells, and spermatogenesis were affected in the early stages of treatment, full spermatogenesis was restored by the end of the injection series. Three additional rats received a 10-day series of injections at the same dose as the other groups; although some morphologic changes occurred, function was not affected at the end of the series. When examined by X-ray after injection of a contrast medium, the testes of another rat given a single dose showed slight enlargement of the lymphatic channels with hemorrhages in some areas. The author [122] concluded that no irreversible damage resulted from any of the test conditions.

In 1972, Waltschewa et al [123] reported the effects of nickel sulfate on the testes of rats. Thirty 5-month-old male albino rats were administered, through an esophageal tube, nickel sulfate at 25 mg/kg daily for 120 days. Ten similar males served as controls. At the end of the treatment, the rats were caged for 24 hours with female rats in estrus, and the number of pregnancies was subsequently determined. The male rats were killed, and their testes, liver, and kidneys were microscopically examined.

Examination of liver sections showed increased lactate dehydrogenase concentration, decreased DPN-diaphorase concentration, and signs of degenerative cell damage. Degenerative cellular changes were also noted in the convoluted tubules of the kidneys. The authors [123] stated that changes in the liver and kidneys were relatively slight and noncharacteristic. Testicles of the treated rats were smaller than those of the controls. The findings from microscopic examination included interstitial cell proliferation and transparent vessel walls. The number of spermatozoa and their precursors in the testicular canaliculi was reduced. Succinodehydrogenase and steroid 3-beta-dehydrogenase concentrations in the testes were decreased. The authors [123] reported that none of the female rats placed with treated males were impregnated; three of six females mated successfully with control males. The authors concluded that nickel sulfate selectively damaged the testes of rats, which resulted in inhibition of spermatogenesis leading to a loss of procreative capacity.

Kar and Sarkar [124] reported in 1960 on their investigation of the effects of nickel on the action of testosterone and estrogen. Gonadectomies were performed on 18 male and 18 female albino rats. After a 15-day period to permit total regression of the accessory genital organs, 12 male rats were given im injections of 62.5 μg of testosterone propionate daily for 4 days, and 12 female rats were given the same dose of estradiol dipropionate. Six rats of each group also received nickel acetate subcutaneously at 0.04 millimole/kg/day for the same period. A three-group comparison was thus provided for each sex: (a) castrate only, (b) castrate with sex hormone, and (c) castrate with sex hormone and nickel acetate.

The animals were killed the day after completion of treatment. The seminal vesicles, ventral prostate, levator ani muscle, and uterus were weighed. Nickel acetate appeared to inhibit the androgenic action of testosterone, since the seminal vesicle, ventral prostate, and levator ani muscle of the nickel-treated males weighed less than those of males treated with testosterone only. The administration of nickel acetate approximately doubled the uterine-weight response to estrogen. These effects of nickel acetate on hormone action could be attributed either to alteration in the relative rate of metabolism of testosterone and estrogen or to interference with the sensitivity of the target tissues to the hormones.

Kamboj and Kar [125] investigated the effects of nickel nitrate injected into one testis of rats and subcutaneously in mice. Six albino rats received single injections of 0.08 millimole of nickel nitrate/kg in the left testis and of sterile distilled water in the right testis. Nine Swiss mice were injected subcutaneously with a 0.2 ml solution of nickel nitrate daily for 30 days, resulting in a total nickel nitrate dose of 0.08 millimole/kg. Eighteen control mice received distilled water subcutaneously. The rats were killed 2 or 7 days after the single injection and the mice were killed 2, 7, or 30 days after their last injection. Testes of both species were weighed and examined microscopically. A reduction in the weight of the treated testes was observed in treated animals of both species but was more marked in rat testes, which weighed half as much as untreated testes at 7 days. Treated rat testes had focal necrosis and hemorrhage. Cellular exfoliation and lysis occurred in the cellular elements in 2 days; at day 7, indications of regeneration of the interstitium were noted. No effect on spermatozoa in

the ductus deferens was observed in rats. Treated mice had no necrotic changes in the interstitial tissue of the testes. However, shrinkage of the tubules and spermatogenic arrest at the primary spermatocyte or spermatogonial stages were noted.

Malaviya and Saraswat [126] studied the effects of nickel chloride on the reproduction of guinea pigs. Male guinea pigs, two at each dose, were injected subcutaneously in the intrascapular region with nickel chloride at doses of 1, 0.001, or 0.0001 mg/kg daily for 15 days. The test animals and a similar group of controls were then mated with fertile females. The females mated to the test animals did not differ from those mated to the controls in the period of gestation, number of litters or offspring, weight of offspring, or offspring development.

(d) Other Effects

(1) Acute Toxicity

The LD50's for some inorganic nickel compounds in several species of animals have been determined. The results are listed in Table III-15 at the end of this chapter. Initial signs of toxicity following the ip injection of nickel acetate into rats and mice included diarrhea, respiratory difficulty, and lethargy [127]. Intestinal adhesions with whitish mucous layers covering the viscera were noted upon microscopic examination [127]. In rats, oral LD50 doses of nickel chloride produced depression of the nervous system, edema of the mucous membranes of the mouth and nose, viscous transparent diffusions from the oral cavity, hyperemia of the nose and outer ear, lacrimation, bleeding from the nose, and diarrhea in rats [128].

(2) Respiratory System

Belobragina and Saknyn [129] reported in 1962 the results of their studies with dust obtained from nickel production areas. Samples of dust were administered intratracheally to 81 white rats at doses of 50 mg/animal. The animals were killed, and their lungs were examined microscopically, 3, 6, 9, or 12 months after treatment. No controls were reported. Dust from the roasting shop, which contained 33.4% nickel oxide, 31.0% nickel sulfide, silicon dioxide, and iron and aluminum oxides, caused high initial mortality (22 of 37 animals died in 5 days); after 6 months, connective tissue nodes were formed and slight sclerosis was present. Another dust, from the electric furnace shop, which contained 95% nickel oxide, produced diffuse sclerosis after 6 months. Rats were also exposed to this dust at a concentration of 80-100 mg/cu m in an inhalation chamber 5 hours/day for up to 12 months. After 6 months, areas of emphysema began to appear, and moderate sclerosis was present after 12 months. For both dusts, hyperplasia of the lungs and peritracheal lymph nodes was observed. According to the authors [129], intratracheal and inhalation exposures to nickel oxide dusts produced similar results.

Selivanova and Ponomarkov [130], in 1963, reported the effects of nickel powder inhalation on dogs. Five dogs were exposed for 10 minutes/day for up to 6 months to nickel powder at a concentration of 5-6 mg/cu m. The animals were kept for observation up to 19 months. No changes were noted in the weight or general condition of the dogs. However, within 2-3 months of the initiation of the exposures, the blood showed a decrease in leukocyte counts, primarily in neutrophils. Necropsy of one dog which died early (time not specified) showed nickel particles

evenly distributed in the lung tissue; nickel was also present in liver and kidney tissues. In two dogs examined 12 months after the beginning of the experiment, the nickel in the lungs was combined into conglomerates around the bronchi and vessels, and there were indications of an interruption in blood flow in the small vessels of the lungs. In two dogs observed for 19 months, pulse and respiration rates were consistently increased. By the end of the 6-month exposure, slight fluctuations in the electrocardiogram were noted, and these increased in the postexposure period, indicating gradual development of cardiac insufficiency. Microscopic examination showed the development of coarse-fiber tissue. The authors [130] concluded that pneumosclerosis had gradually developed in the lungs after inhalation of nickel powder causing cardiac insufficiency. Since controls were not used and the ages of dogs were not given, it is not possible to determine if the effects were the result of nickel exposure.

In 1972, Bingham et al [131] reported pulmonary responses of Wistar rats to inhalation of respirable aerosols (less than 1 μm in diameter) of nickel chloride and nickel oxide. The average concentrations, measured as nickel, at which the animals were exposed were 109 μg/cu m for nickel chloride and 120 μg/cu m for nickel oxide. Controls were exposed to filtered air. The period of exposure was 12 hours/day, 6 days/week, for up to several months. After the last exposure, the lungs were excised and cells collected by lavage and counted. After 2 weeks of exposure, the average numbers of cells were 3.5 million/g of lung in controls, 5.1 million/g of lung in nickel chloride-exposed rats, and 9.8 million/g of lung in nickel oxide-exposed rats. After 4-6 weeks of exposure, the number of cells washed from nickel oxide-exposed animals had increased to more

than 11 million/g of lung. Findings from microscopic examinations of lungs of rats exposed to nickel oxide for 2 weeks included accumulation of macrophages in the alveolar spaces, hypersecretion in the bronchial epithelium, and lymphocyte infiltration of the alveolar walls and perivascular spaces. After longer exposures, lymphocytic infiltration and the number of intraalveolar macrophages were found to have diminished, and focal thickening of the alveolar walls was also observed. Findings in rats exposed to nickel chloride included mucus secretion, hyperplasia, and peribronchial lymphocytic infiltration. Alveolar macrophages, however, were less abundant than in the lungs of rats that had inhaled nickel oxide. Washings from the lungs of rats exposed to both compounds, and particularly nickel chloride, were viscous and cloudy. Bingham et al [131] concluded that inhalation of nickel oxide and nickel chloride at concentrations as low as 0.1 mg/cu m induced some pathologic changes and produced an increase in the number of alveolar macrophages.

An unpublished report by Clary [132] outlines preliminary results following exposure of rats to airborne nickel chloride at a concentration of 1 mg/cu m, measured as nickel. Thirty exposed and 30 control male rats were used. Guinea pigs were also used but results were not included in this preliminary report. Exposures were 5 days/week for 3 months, at which time half of the animals were killed, or for 6 months. The exposure and control groups showed no difference in serum biochemistry, body weights, or liver glucose levels. Findings at necropsy for both the animals killed at 3 months and 6 months included increased lung weights and nickel accumulation in lungs and kidneys. Most control animals had mild to moderate bronchitis and bronchiolitis at 3 months; in addition, 7 of 15 had

moderate fibrosis (average score=1+). Exposed animals had about the same incidence and severity of bronchitis and bronchiolitis as control animals; however, fibrosis in the alveolar ducts was more marked (average score=3+) and occurred in all 11 examined animals. Signs of irritation in the exposed animals included proteinaceous material in alveolar spaces and proliferation of type II granular pneumocytes. No signs of metaplasia were observed. The lungs of the control and exposed rats killed at 6 months were similar to animals killed and examined at 3 months. In the exposed rats there were slightly greater numbers of foamy macrophages and more interstitial fibrosis than observed in controls. In both cases, examination of the liver, kidneys, or pancreas revealed no lesions.

In 1972, Wehner and Craig [133] reported on the retention of nickel oxide in the lungs of Syrian golden hamsters. Eight 3-month-old male hamsters were exposed 6 hours/day, 5 days/week, for 3 weeks at a mean concentration of 39 mg/cu m. The animals were killed 4 days after the last exposure, and the lungs were analyzed for nickel; assuming a mean inhalation volume of 60 ml/minute, the authors calculated that 19.3% of the theoretical dose was retained in the lungs. In a final report on the study [134], the lungs of these animals were described as having mild to significant interstitial inflammatory reactions, congestion, and emphysema. Liver cells showed mild degenerative changes; kidneys were normal.

Wehner and Craig [133] also exposed 34 hamsters of both sexes to nickel oxide at a concentration of 61.6 (SD=30.3) mg/cu m. Seventeen of these animals were also exposed to cigarette smoke. The animals were exposed 4 hours/day, 5 days/week, for 3 months. Control animals were mentioned but not described. From 16 animals that were killed 4 days after

the last exposure, the authors calculated that 19.7% of the theoretical nickel oxide dose was retained in the lungs of the hamsters. The final report [134] provided details of a microscopic examination of the lungs of these animals. No differences were reported between animals exposed to nickel oxide and those exposed to nickel oxide and smoke. Nickel oxide particles were phagocytized and concentrated in the macrophages in massive accumulations throughout the lungs. Blood cells accumulated in several parts of the lung interstitium, and there were occasional points of hemorrhage and edema. The kidneys showed mild glomerular congestion, hypertrophy of the endothelial lining of Bowman's capsule, and occasional proteinaceous casts. The livers were normal.

(3) Endocrine and Enzyme Systems

Kadota and Kurita [135] investigated the effect of nickel chloride on the blood glucose level of rabbits. They reported that 7 of 11 rabbits injected iv with nickel chloride at 10 mg/kg showed transient hyperglycemia 1-4 hours after injection. All seven rabbits that received 15 mg nickel chloride/kg exhibited pronounced hyperglycemia, but the glucose level returned to normal within 24 hours. Histologic examination of pancreases taken from four rabbits 1 hour after injection of nickel chloride at 15 or 20 mg/kg indicated alpha-cell destruction, degranulation, and some necrosis of beta cells.

Mikhaylov et al [136] exposed 64 male white rats to nickel dust which contained sulfides (20.9%) and oxides (55.4%) of nickel. The animals were exposed at 70 mg/cu m, 4 hours/day, for 4 months. After 3-4 months, body weights were significantly less than those of control animals. At the same time, decreases in the concentrations of sugar and glycogen in the blood

and liver were noted. The authors [136] also reported that intratracheal administration of this dust in doses of 40-180 mg/kg produced liver congestion and necrobiotic areas.

Horak and Sunderman [137] have reported the effects of nickel chloride, administered ip, on the plasma glucose and serum insulin concentrations of female Fischer rats. Nickel chloride produced hypoglycemia in fasting rats 2 hours after injection of 1-17 μmol/kg (about 0.13-2.2 mg/kg) and hyperglycemia 0.5 and 1.0 hours after injection of 17-85 μmol/kg (about 2.2-11.0 mg/kg). The authors [137] also reported the effect of nickel chloride on plasma concentrations in hypophysectomized or adrenalectomized rats (compared to effects in sham-hypophysectomized or sham-adrenalectomized rats). The hyperglycemic responses to injections of nickel chloride were suppressed but not completely inhibited by the adrenalectomy. Adrenergic blockade did not affect the hyperglycemic influence of nickel. Exogenous insulin (1-5 units/kg) antagonized the hyperglycemic effect of nickel chloride (68 μmol/kg or 8.8 mg/kg), but the effect was not entirely overcome. Increases in serum immunoreactive insulin levels were observed 1 hour after injection of nickel chloride at a concentration of 34 μmol/kg (about 4.4 mg/kg) and the increases persisted 0.5-3 hours after the injection of 68 μmol/kg. The authors [137] concluded that the hyperglycemic effects of nickel were antagonized by exogenous insulin and suppressed by adrenalectomy and hypophysectomy, and that the insulinogenic response of the pancreas to hyperglycemia was unimpaired by nickel. These observations, along with the finding that adrenergic blockade was ineffective against nickel-induced hyperglycemia, suggest that this hyperglycemia may not be caused by stimulation of the adrenal glands.

Clary [138] reported the effects of intratracheally injected nickel on glucose metabolism in rats. Twenty male albino rats were given an oral dose of 600 mg of 14C glucose and challenged with 0.5 mg of nickel. Five rats were killed immediately after the injection and others were killed hourly up to 3 hours after the injection. Twenty-four additional rats, in groups of three, were given various combinations of 0, 0.25, 0.5, or 1 mg of nickel and 0, 0.25, 0.5, or 1.0 units of insulin; the animals were then killed 30 minutes after the injection. In the initial test, glucose and 14C concentrations in serum increased after nickel administration, whereas insulin and liver 14C concentrations decreased. In the second test, 0.5 mg of nickel given with 0.25 units of exogenous insulin had no effect on serum glucose concentrations. Other combinations of nickel and insulin resulted in increased or decreased serum glucose levels, depending on the nickel-to-insulin ratio. The author [138] concluded that the increase in glucose probably reflected the influence of nickel on the production or secretion of insulin. He hypothesized that nickel did not influence insulin directly, but rather affected pituitary hormone secretions which control insulin concentrations.

LaBella et al [139] observed that iv injection of nickel (as nickel chloride) resulted in a decrease of serum prolactin, a hormone secreted by the pituitary. A single injection of nickel chloride containing 100 μg or 200 μg of nickel was administered to urethane-anesthetized rats which had been treated with chlorpromazine. Prolactin concentrations in controls after 30 minutes averaged 113 ng/ml of serum, whereas prolactin concentrations were 72 ng/ml in rats treated with 100 μg of nickel and 66 ng/ml in rats given 200 μg. LaBella et al [140] also reported in vitro

results showing that nickel ion inhibited prolactin release from incubated bovine pituitary fractions.

Sobel et al [141] administered a nickel chloride saline solution to 18 guinea pigs by ip injection at a dose of 4 μmol/100 g (about 5 mg/kg). Eighteen controls received an injection of saline only. Urinary corticoid excretion in guinea pigs injected with nickel chloride was almost twice that of controls.

Yeliseyev [142] exposed 50 male white rats to a hydroaerosol of nickel chloride, 24 hours/day for 6 months at nickel concentrations of 500, 5, 1, or 0.2 μg/cu m. A control group was exposed to an aerosol of tap water. Inhalation of nickel chloride at 500 and at 5 μg/cu m resulted in a reduction of the iodine-fixing function of the thyroid gland relative to controls. No changes were observed with concentrations of 1 or 0.2 μg/cu m. Yeliseyev [142] also investigated the effect of nickel chloride on an unspecified number of rats given doses of about 5, 0.5, 0.05, or 0.005 mg/kg/day in their drinking water. A control group received tapwater only. Oral ingestion of nickel at 5 or 0.5 mg/kg resulted in reduction of the iodine-fixing function of the thyroid, as compared to controls, whereas a dose of 0.05 mg/kg caused an increase in this function during the 1st month, a return to normal at the 2nd month, and a decrease at the 3rd and 6th months. No change was observed at a dose of 0.005 mg/kg. The author concluded that inhalation was a more dangerous route of administration than oral ingestion, and that oral ingestion of nickel at 0.05 mg/kg of body weight and inhalation at 0.005 mg/cu m were isoeffective, threshold doses.

Iodine retention in the thyroid has also been studied in dogs exposed to nickel powder [130]. Five dogs were exposed by inhalation to nickel

powder at a concentration of 5-6 mg/cu m, 10 minutes/day, for 6 months. Radioactive iodine (131I) was used to measure the rate and extent of iodine retention. The normal 24-hour accumulation of 131I in the thyroid was 22% of the administered dose. Three months after exposure to nickel began, 35% of the iodine administered was retained; after 7 months, 41%; and after 12 months, 44%. At the end of the 19-month study, retention had returned to normal.

Shvayko and Tsvetkova [143] reported the influence of orally administered nickel on the thyroid function of white rats. Nickel chloride, in nickel doses of 1, 25, and 100 mg/kg/day, was mixed with the food given to 89 male rats over a period of 3.5-4 months. The rats receiving 1 mg/kg showed greatly decreased iodine uptake as measured with radioactive iodine. Iodine uptake was increased at the 25 mg/kg dose but gradually decreased at the 100 mg/kg dose.

Apparent conflicting trends of iodine uptake in these studies [130,142,143] and their implications to thyroid function need clarification before conclusions can be drawn on the appropriateness of these studies to an occupational health standard.

(4) Skin

The potential for skin absorption of inorganic nickel has not been thoroughly studied. In 1960, Choman [144] published the results of autoradiographic studies on the percutaneous absorption of nickel chloride by two rats. The concentration of the nickel chloride solution was 0.1% and the pH was 6.5. Labeled 63-nickel chloride was applied to shaved areas on the backs of the rats and left for 60 minutes. The animals were killed, the skin at the application site was removed, and autoradiographs were

prepared. Four hours later, radioactive nickel chloride was applied to another section of skin removed from the dead rats, and after 60 minutes, an autoradiograph was prepared. Nickel chloride was absorbed only to a depth of 25 μm in both animals whether applied before or after death. The penetration depth of 25 μm suggested to the author [144] that nickel chloride contacted only the first layer of the epidermis and was not absorbed through the skin. Pretreatment of rat skin with a detergent, sodium lauryl sulfate, facilitated nickel chloride penetration to an average depth of 425 μm in living rats.

Norgaard [145] measured the amount of radioactive nickel absorbed through the dorsal skin of guinea pigs and rabbits in 24 hours. The fur was removed by chemical depilation from a 5- x 5-cm area from each of two animals of both species, and radioactive nickel (57Ni, apparently as a solution of nickel sulfate) was applied. Following application, excess moisture was evaporated, and the area was covered. The animals were killed after 24 hours, and the radioactivity in the urine, blood, liver, and kidneys was measured. The amounts of activity in all four tissues varied, but the amounts were not convertible to absolute amounts of nickel in the tissues, as only samples of each tissue were taken. This study showed that nickel applied to the skin of guinea pigs and rabbits was absorbed and distributed.

Nickel chloride (30% in aqueous solution) has been tested for acute skin irritation potential [146]. Six albino rabbits were given 0.5 ml of the solution on intact and abraded skin. The sites were occluded, and the development of erythema and edema was scored at 4, 24, and 72 hours after the beginning of exposure. A slight erythema was noted at the abraded

sites after 4 hours of exposure; all other observations were negative. The primary irritation score was 0.2, meaning that nickel chloride in this standard test was not corrosive.

Kolpakov [147] studied the toxicity of nickel sulfate solution applied to the dorsal skin of white rabbits. The fur was removed by chemical depilation from a 10- x 10-cm area of each animal's back, and a 20% solution of nickel sulfate was applied on a gauze compress. Five rabbits were treated on intact skin; in six rabbits, the epidermis was destroyed with fine emery paper, and nickel sulfate was applied to three, with three kept as controls; and, in the last group, an unspecified organic solvent was first applied for 6 days, followed by nickel sulfate (three animals) or physiologic saline (three animals). After removal of the compress, sections of skin, liver, kidneys, and lungs were removed and examined for the presence of nickel. In the rabbits with intact skin, no nickel was detected in the liver, lung, or kidney sections and there were no signs of toxicity. In the animals whose skin had first been abraded, toxic signs (convulsions and salivation) appeared within 2-3 hours, and all the animals died within 7-10 hours. Nickel was detected in the skin, liver, kidneys (particularly in the medullar portion), and lungs. No effects were noted in the control animals. In the rabbits pretreated with an organic solvent, death occurred within 7 hours, and nickel was again detected in all tissue sections. No deaths occurred in the control rabbits treated with organic solvent only.

(5) Kidneys

In 1975, Gitlitz et al [148] reported the results from ip injection of nickel chloride in female albino Fischer rats. Nickel chloride in sterile sodium chloride solution was injected in doses of 4 ml/kg containing 2, 3, 4, or 5 mg of nickel. Each dose level was tested on groups of five or six rats, and five control animals were injected with the vehicle only. Within 2 days of the injection, all the test doses caused significant increases ($P<0.01$) in the urinary excretion of protein, ie, proteinuria, which is evidence of kidney damage. Amino acid excretion was not altered at the 2 mg/kg dose, but histidine, 1-methylhistidine, and sarcosine excretion was affected by a 3-mg/kg injection. In doses of 4 and 5 mg/kg, nickel chloride affected the excretion of all acidic, neutral, and basic alpha-amino acids except aspartic acid. Plasma levels were monitored to determine whether increased urinary amino acid excretion was a result of increased plasma levels, but no increases were noted. Pathologic alteration of the renal tubules were found in one of five rats killed 48 hours after ip injection of 4 mg of nickel/kg. Electron microscopy in these rats also showed fusion of the foot processes of epithelial cells in renal glomeruli. This effect appeared to be reversible.

(6) Eyes

A commercial nickel chloride product has been tested in rats for eye irritation potential [146]. The proprietary formula was reported to contain 30% nickel chloride in aqueous solution. Three albino rats were given 0.1 ml of the solution in an unwashed eye; the other eye served as a control. The development of irritation was scored according to the tissue involved (cornea, conjunctiva, or iris) and the intensity of irritation.

The irritation score for nickel was 19.6 out of a possible 110.0 points. This score was classified as mildly irritating.

(e) Metabolism

Wehner and Craig [133] reported the results of pulmonary deposition and clearance studies with nickel oxide in hamsters. Ninety-two 2-month-old male Syrian golden hamsters were exposed to nickel oxide at 73.5 mg/cu m for two consecutive 7-hour periods. Immediately after exposure, four randomly selected animals were killed, and tissues from the lungs, liver, kidneys, and gastrointestinal tract were analyzed for nickel by atomic absorption spectrophotometry. Subsequent groups of four animals were killed at increasing intervals, the last group 155 days after the exposure. Nickel oxide was only slowly removed from the lungs; by the 6th day after exposure, more than 70% of the nickel oxide was still present. Only small amounts of nickel oxide were found in the liver, gastrointestinal tract, or kidneys at any time.

Wehner and Craig [133,134] studied the uptake of nickel oxide from the gastrointestinal tract of hamsters. Nickel oxide, 5 mg in suspension, was administered by gavage to six hamsters. Twenty-four hours later, the animals were killed, and tissues were analyzed for nickel by atomic absorption spectrophotometry. There was no increase over control values in the nickel content of the lungs, liver, kidneys, or carcass of the experimental animals.

Clary [138] studied the distribution of radioactive nickel chloride administered intratracheally to 30 male albino rats. A 63-nickel chloride solution containing 1 mg of nickel was injected into each animal, and the amount of nickel in various organs was determined 6 hours after injection.

The authors stated that the kidneys showed the greatest concentration of nickel/g of tissue, followed by the lungs, adrenals, liver, pancreas, spleen, heart, and testes. Other tissues had only a very low nickel content. All tissues had much lower levels 24 hours after injection. By 72 hours, 90% of the nickel dose had been excreted, mainly in the urine (75%).

Ho and Furst [149] compared oral and ip administration of radioactive nickel chloride in rats. Female Fischer-344 rats were given doses of 9.12, 2.28, or 0.57 mg of nickel ion by stomach tube or ip injection. Regardless of the quantity of nickel given orally, the entire amount was excreted in 48 hours, 3-6% in the urine and the rest in the feces. Animals given nickel chloride by ip injection excreted only 1-2% of the nickel in the feces and the rest in the urine. Maximum excretion was reached 1 hour sooner in ip-injected rats than in the orally treated animals.

Sunderman [150] reported the tissue distribution of nickel in rabbits after iv injection of radioactive nickel chloride. Rabbits killed 2 hours after a single injection of 4 μmol/kg (about 0.52 mg/kg) had the greatest concentration of nickel/g of tissue in the kidneys, followed by the pituitary, serum, lungs, heart, and liver, with the lowest concentration in the hypothalamus. Rabbits killed 24 hours after the last of 34-38 daily injections of 75 nmol/kg/day (about 9.7 μg) showed similar results, with the highest concentrations in the kidneys and the pituitary, but blood levels were reduced and spleen levels were elevated.

Sunderman and Selin [151] investigated the metabolism of radioactive nickel chloride in rats after iv injection. An average of 86.7% of the administered dose was excreted in the urine within 24 hours, and only 0.5%

was excreted in the feces. After 4 days, 92.8% of the injected dose had been recovered.

Chen et al [152], using colorimetric analysis for nickel determinations, reported that rats injected im with 60 mg of nickel acetate excreted 95% of this compound in the urine within 2 weeks. Rats injected with 60 mg of nickel powder, however, still excreted nickel after 6 weeks.

Wase et al [153] injected radioactive nickel chloride into mice and determined the distribution of nickel. Fifty-six adult male mice were administered 102 μg of nickel as nickel chloride by ip injection. Most of the nickel was excreted in the urine and feces by 8 hours after the injection, with fecal excretion predominating. The kidneys contained the highest concentration of nickel, followed by the lungs and plasma. The authors [153] also noted that, at 72 hours after injection, the lungs still retained almost 40% of the nickel initially found there.

(f) Essentiality of Nickel

A growing body of evidence suggests that nickel has an essential physiologic role in animals. According to Sunderman and coworkers [154] in 1972, this evidence included (1) the finding that serum nickel concentrations appeared to be controlled within relatively narrow ranges [155,156]; (2) identification of a metalloprotein which contained nickel [156-158]; (3) the observation that serum nickel concentrations are altered by several human diseases [159-161]; and (4) results of a preliminary study of induced nickel deficiency in chicks [162-164]. Nielsen [165,166] has reviewed the literature on the essentiality of nickel and concluded that nickel met the requirements for essentiality. In 1975, it was concluded in the NAS-NRC report [3] that nickel partially satisfied established criteria

for essentiality. Unequivocal demonstration that nickel deprivation produced abnormalities that could be cured or prevented by nickel administration was still lacking [3].

Since the publication of these reviews, Nielsen et al [167] have reported results which show additional support for nickel essentiality in chicks. Nielsen et al [168], as well as Schnegg and Kirchgessner [169], have demonstrated, in three-generation studies, nickel deficiency in rats. Schnegg and Kirchgessner [169] stated that the daily nickel requirement for the normal growth of rats was presumably near 50 ppb. This information neither supports nor refutes the premise that a safe exposure level for dermal contact with or inhalation of nickel can be determined, and it should be interpreted as the conclusions of the individual authors rather than those of NIOSH.

Correlation of Exposure and Effect

No reports on the acute toxicity of inorganic nickel in humans have been found. LD50 data in animals for several nickel compounds are summarized in Table III-15 [127,128,136,137,170-174].

Studies of electrolysis workers in nickel refineries in the USSR have shown erosions [26,49,50] and perforations [49,50] of the nose and impairment or loss of the sense of smell [49]. In one study [49], it was noted that symptoms changed in number and kind with duration of employment, but erosions of the nasal septum did not tend to change with age or progress to perforations over the 1.5-year study period. Similar findings have not been reported in other nickel refineries. In a Norwegian refinery, however, atypical epithelial changes were noted in 17% of the

nasal tissue samples removed from nickel workers by biopsy [72].

Some workers exposed to nickel, particularly those who had skin contact with nickel solutions, developed dermatosis [27-29]. The only recent report giving the incidence of occupational dermatosis for nickel workers was a study from the USSR in which 1.8-6.2 cases/100 workers were observed [26]. In a 1926 report [18], dermatosis in a nickel refinery was most prevalent in workers handling nickel salts and solutions; heat and humidity were contributing factors. A recent report [27] also noted that more cases of nickel dermatitis were observed in workers around heated electroplating tanks than were observed in workers employed in cold plating.

Cuts and abrasions [28,29] and the use of degreasing agents [28] have been observed to be predisposing factors in the development of dermatitis. Additional evidence is available from animal studies. In rats administered nickel chloride on intact skin, nickel penetrated only to the first layer of the epidermis; on skin treated with sodium lauryl sulfate, nickel penetrated much further [144]. Nickel was not found in the organs of rabbits administered nickel sulfate on intact skin, but if the skin was first abraded or treated with an organic solvent, nickel was found in the liver, kidneys, and lungs [147].

Poor work practices, such as the failure to wear gloves, have been cited as contributing to the development of dermatitis [27,29]. Once sensitivity to nickel has been developed, it has persisted, and workers have had to be transferred to areas where additional contact with nickel was unlikely [29,30].

Asthmatic disease has been reported in persons with nickel dermatitis [52,53]. In one case, eosinophilia was diagnosed [53]. Although these case reports are insufficient to permit any conclusions concerning occupational exposure to nickel, it appears that a few persons who become sensitive to nickel may develop lung complications.

Inhalation studies in animals have demonstrated adverse lung effects from exposure to inorganic nickel. Rats exposed to nickel chloride at a concentration of 0.1 mg of nickel/cu m for 12 hours daily for several weeks developed hyperplasia and showed peribronchial lymphocytic infiltration and mucus secretion [131]. Rats exposed to nickel chloride at 1 mg of nickel/cu m for 6 months had increased lung weights, and nickel appeared to exacerbate preexisting lesions [132]. Sclerosis has been reported in dogs [130] and rats [129], although no definite conclusions could be drawn from these experiments because of the lack of controls.

In parts of several long-term inhalation studies designed to assess the carcinogenicity of nickel and its compounds, other effects were also noted. Hamsters exposed to nickel oxide at a concentration of 39 mg of nickel/cu m for three weeks developed inflammation and congestion of the lungs and emphysema [134]. The lungs of rats and guinea pigs receiving lifespan exposure to elemental nickel at an average concentration of 15 mg/cu m had excessive adenomatoid lesions; in rats, the majority had chronic paranasal sinus inflammation and ulceration; and in guinea pigs, edema, hemorrhage, and hyperemia were common [82]. In a similar experiment, hamsters showed little effect from exposure to metallic nickel [83]. Rats exposed to nickel subsulfide at a concentration of about 1 mg/cu m for approximately 80 weeks had abcessed, consolidated lungs with

varying degrees of hyperplasia or metaplasia [78]. In humans, only two reports of pneumoconiosis were found [54,55], and they are inadequate to assess the possibility of adverse lung effects in humans from exposure to inorganic nickel. Considering the number and degree of changes observed in animal studies, especially when these resulted in cancer, a more thorough study of respiratory organ effects other than cancer is needed in humans.

Nickel chloride injected ip has caused proteinuria and aminoaciduria in rats [148]. Nickel may accumulate in human kidneys [175] and it has been shown to accumulate in the kidneys of animals [138,150]. These studies are only suggestive of kidney effects and further studies are needed to determine if adverse effects of exposure to inorganic nickel may result in functional changes in the kidneys.

Animal exposures to inorganic nickel have produced a variety of endocrine [130,138] and enzymatic [136,138] changes. The iodine-fixation rate [130] and the levels of insulin [138], glucose [137,138], and glycogen [136] were reportedly affected. These effects are difficult to extrapolate to humans, since none of these effects has been investigated in workers exposed to nickel.

Inorganic nickel has not been adequately tested for ocular toxicity. Nechiporenko [67] has reported eye damage in electrolysis workers, but the extent of exposure was not reported. An animal study [146] also suggests that nickel chloride (30% in proprietary solution) may be mildly irritating to the eyes.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

(a) Carcinogenicity

Epidemiologic studies indicate that workers engaged in operations in which nickel is processed have increased risks of developing cancer of the nose [35,37,39-41,43,44,47], lungs [35,37,39-41,43-46], larynx [41,43], and possibly the kidneys [44 and E Pedersen, written communication, December 1976]. Environmental data on the concentrations of airborne nickel or on the chemical compositions of nickel compounds to which workers were exposed were not provided in any of the epidemiologic studies on nickel workers. However, based on estimates of the concentrations of airborne nickel in the past [41] and information on the processes used to refine nickel [10,23,36,41], it appears that workers who developed lung or nasal cancer were exposed to mixtures of many different nickel compounds, and that the risks of developing lung and nasal cancer were greatest for workers employed in operations which had the highest concentrations of airborne nickel.

An increase in the number of deaths from nasal cancer in nickel workers was first noted in 1932 in workers at a nickel refinery in Clydach, Wales [21]. Epidemiologic studies by Doll et al [39,40] have shown that the risks of death from lung and nasal cancer were highest in workers first employed at the Clydach nickel refinery before 1925 and have decreased substantially for workers beginning employment in subsequent years. In the most recent study, Doll et al [40] reported that, as of the beginning of 1972, the O/E ratio of deaths from lung cancer had decreased from 7.0:1 in workers first employed before 1925 to 1.9:1 in workers first employed after 1925. The O/E ratio of deaths from nasal cancer was 329:1 for workers

first employed before 1925. No deaths from nasal cancer have been identified in workers first employed after 1925, but nasal cancer was listed as an associated cause of death for one worker first employed in 1929 [40].

In epidemiologic studies, Hill [35] and Doll [37] found that process workers were more likely to die from lung or nasal cancer than nonprocess workers. In one study [37], the risks of death from lung and nasal cancer were significantly greater in both process and nonprocess workers than in the general population of England and Wales. Doll [37] reported that, as of 1956, the O/E ratio of deaths from nasal cancer was 297:1 in process workers and 119:1 in nonprocess workers, and the O/E ratio of deaths from lung cancer was 7.1:1 in process workers and 3.4:1 in nonprocess workers.

In 1958, Williams [38] found that concentrations of nickel, but not of arsenic, were elevated in the lungs of two Clydach nickel refinery workers. The concentrations of nickel were 90 and 120 ppm of dry tissue, compared to an average nickel concentration of less than 5 ppm in the lungs of two comparison groups. In contrast, the concentrations of arsenic were less than 0.2 ppm in the lungs of the two nickel workers and the lungs of both comparison groups.

In a study of 507 workers employed in one department at the Clydach refinery for 15-25 years, Morgan [36] found that deaths from nasal cancer and lung cancer occurred most frequently in calciner furnace workers, especially those who cleaned the flues, and in copper sulfate process workers, followed by other furnace workers, crushing and grinding department workers, and workers transporting matte to the Mond (carbonyl) process area. Morgan [36] reported that Clydach nickel refinery workers

began to wear gauze masks in 1922; Inco recently reported that workers always wore double gauze masks [41]. The efficiency of these double gauze masks ranged from 70 to 95%, but the masks were less efficient for smaller-sized particles (less than 4 μm) [41]. The most recent epidemiologic study by Doll et al [40] has shown that the risk of death from nasal cancer in Clydach nickel refinery workers has decreased with the year of first employment more rapidly than the risk of death from lung cancer. This finding indicates that larger particles were probably associated with the development of nasal cancer and smaller particles were probably associated with the development of lung cancer. The gauze masks first worn by Clydach workers in 1922 appear to have been the most significant factor in reducing worker exposure to airborne dust at this refinery.

No information was found on specific nickel compounds to which Clydach workers were exposed. However, a substantial amount of information was available on processes used to refine nickel and how they were changed over the years [10,23,36,41]. The assumption that workers were exposed to dusts and fumes of materials present in the operations in which they were employed is reasonable and permits some qualitative estimates of the nickel compounds involved. Quantitation is not possible, since nickel sulfides, escaping into the air as burning particles, were probably at least partially converted to nickel oxides; thus, exposure to nickel oxide may have been greater than the nickel oxide content of the feed material would suggest. Additionally, some contaminants are more volatile than nickel so that higher concentrations of these elements would be possible [41].

Initially, feed material at Clydach was a nickel-copper sulfide matte containing 40-45% nickel [23]. Workers in the calciner sheds were exposed

to dusts from the crushing and grinding of the matte and to nickel sulfides and nickel oxides emitted during the high-temperature oxidation process. Workers extracting copper and transporting the leached matte to the carbonyl sheds were exposed to calcined nickel oxide dusts and, before 1925, to arsenic present in the acid used for leaching [36]. Since material from the carbonyl sheds was returned for reprocessing [41], calciner workers were also exposed to materials added during the process. After 1933, the sulfur content of the feed was decreased, and, in 1945, the feed was changed to nickel oxide [36]. Thus, the forms of nickel to which workers were exposed have varied over the years.

Some monitoring data are available for limited areas of the Clydach refinery. In 1932, changes in calciners appeared to have lowered sulfur dioxide levels from about 25 ppm to about 1.5 ppm, although total dust concentrations remained high [41]. Based on an estimate of 70% nickel in the dusts, there were roughly 20 mg of airborne nickel/cu m in the calciner sheds in 1949. However, wide variations were reported [41] and employee exposures cannot be estimated. No data were available to estimate worker exposure in the pre-1925 conditions and the percentages of contaminants in dusts are not available.

In 1950, Loken [42] reported three cases of lung cancer in workers employed at a nickel refinery in Kristiansand, Norway, where matte from nickel sulfide ore was processed [10]. Two of the workers had been employed in the roasting kiln area and the third had worked in shearing nickel and as a foreman of an unspecified department. The concentration of nickel in the lungs of one of the affected workers from the roasting kiln area was reported to be quite high, 2.8 mg/g of dry tissue, although the

worker had not worked at the refinery for 10 years prior to the determination.

An epidemiologic study by Pedersen et al [43] has shown that workers in many occupations at the Kristiansand nickel refinery had an increased risk of developing cancer of the respiratory organs. The risk was greatest in roaster and smelter workers and in electrolysis workers, but workers employed in other process departments at the nickel refinery also had an increased risk. In roaster and smelter workers, the O/E ratios of cases were 50.0:1 for cancer of the nose, 10.0:1 for cancer of the larynx, and 4.8:1 for cancer of the lung. In the electrolysis department workers, the O/E ratios of cases were 30.0:1 for cancer of the nose and 7.2:1 for cancer of the lung; no cases of cancer of the larnyx were identified. In process workers in other departments, the O/E ratios of cases were 10:0:1 for cancer of the nose, 5.0:1 for cancer of the larynx, and 4.6:1 for cancer of the lung. In nonprocess workers and workers in unspecified processes, the O/E ratios of cases were 20.0:1 for cancer of the nose and 1.5:1 for cancer of the lung; no cases of cancer of the larynx were identified. The risk of developing lung cancer was highest in electrolysis workers employed at the refinery for at least 15 years (O/E ratio of 23.4:1) [43]. In addition, three cases of kidney cancer have been reported in workers at this refinery (E Pedersen, written communication, December 1976). Pedersen et al [43] showed that increased risks of developing cancers of the nose, larynx, and lung were not limited to workers engaged in the roasting and smelting of nickel ore. At the Kristiansand nickel refinery, the risk of developing lung cancer was greater in electrolysis workers than in roaster and smelter workers; the overall risk of developing cancer of the respiratory organs

was significantly greater in all nickel refinery workers than in the general population of Norway. Dust from nickel sulfide or oxide was present in the roasting and smelting departments, while aerosols of nickel chloride and sulfate were predominant in electrolysis areas; nickel oxide and sulfate were found in copper-leaching areas (E Wigstol, written communication, April 1977).

In 1965, Tatarskaya [48] reported two cases of nasal cancer in workers engaged in the electrolytic refining of nickel in the USSR. Epidemiologic studies [58,59] from the USSR suggest that workers engaged in processing nickel oxide ore have an increased risk of death from cancer at several sites. Because of methodologic deficiencies, however, interpretation of these studies [58,59] is difficult. In addition, sulfur was added in the process [10], so no extrapolation of the results can be made to other nickel oxide ore refineries using different processes.

In an epidemiologic study of deaths in workers and pensioners at a nickel refinery in Port Colborne, Ontario, Sutherland [44] found that, between 1930 and 1957, the O/E ratios of deaths in nickel workers were 37.1:1 for nasal cancer and 2.2:1 for lung cancer. Sutherland's study was updated by Inco in 1976 to include deaths that occurred in Port Colborne workers between 1930 and 1974 [41]. In the updated study, the O/E ratios of deaths for these workers were 51.1:1 for nasal cancer, and 1.9:1 both for cancer of the lung and for cancer of the larynx. The occupational histories of 36 workers who had developed nasal cancer and of 90 workers who had developed lung cancer as of June 1976 [41] indicated that deaths from lung and nasal cancer were not limited to calciner, sinter, or cupola furnace workers [41]. Of the 36 workers who developed nasal cancer, one

was employed only in occupations that were not dusty, one was employed only as an electrolysis worker, one was employed only as an anode furnace worker, and three others were never employed as calciner, sinter, or cupola furnace workers. The 90 workers who developed lung cancer were also employed in different occupations at the refinery. The expected number of deaths in each exposure group was not provided, but, based on the occupational histories of these 126 workers, it appears that deaths from lung and nasal cancer in Port Colborne nickel refinery workers were not limited to any one exposure group. In addition, 3 deaths from kidney cancer were identified in a group of 225 electrolysis workers [44], suggesting that these workers may also have an increased risk of developing kidney cancer, since kidney cancer is rare in the general population.

The available information on nickel refining processes [10,23] and limited environmental data [41] were used to estimate the major airborne contaminants at the Port Colborne nickel refinery. Cupola furnace workers handled mixed copper and nickel sulfides. Impure nickel sulfides, containing nickel subsulfide, were oxidized in sinter and calciner furnaces at temperatures of 600-1,650 C [41]. Workers near these furnaces were probably exposed to high concentrations of nickel subsulfide and nickel oxide dust and fume. Area monitoring in the sinter plant at Port Colborne indicated an average total dust concentration of about 340 mg/cu m, estimated to contain about 50% nickel [41]. Since nickel oxide was reduced to nickel in anode furnaces [23], workers near these furnaces were exposed to airborne nickel oxide and possibly to elemental nickel. Electrolysis workers at the Port Colborne refinery were probably exposed primarily to mists of nickel salts and hydrides emitted from the electrolysis tanks,

which contained nickel sulfate and boric acid until the 1940's, when nickel chloride was also added [23], and to other nickel compounds, including nickel metal and nickel carbonate, used in the purification of the electrolytic solution. Some electrolysis workers, particularly those engaged in cleaning anode scrap or operating the desulfurization furnace may have been exposed to high concentrations of nickel. High volume samples taken recently near electrolysis tanks had an average concentration of 0.11 mg/cu m of airborne nickel, although one personal sample had a concentration of 8.13 mg/cu m of airborne nickel [41].

In workers at a sinter plant in Copper Cliff, Ontario, Sutherland [45] found that the risk of death from lung cancer was elevated. One death from nasal cancer was also identified in the sinter plant workers studied by Sutherland. These workers were exposed to dusts and fumes at concentrations similar to those observed in the Port Colborne sinter plant [41]. In an epidemiologic study [46] of deaths from cancer in four other occupational groups at the Copper Cliff nickel smelter complex, Sutherland found that the O/E ratio of deaths from cancer of the respiratory organs was slightly elevated in all four groups combined (converter furnace workers, mill and matte separation workers, underground miners, and copper refinery workers), but it was not significantly greater than in the population of Ontario.

No data on exposures were available for these groups. Recent data suggest that miners are exposed to nickel at concentrations between 6 and 40 μg/cu m and that concentrations of airborne nickel now range from undetectable to 2.8 mg/cu m in the mill area, 0.17-15.3 mg/cu m in the matte separation area, and 0.03-0.2 mg/cu m in the converter furnace area.

Preliminary epidemiologic studies [41,47] suggest that some workers at a nickel alloy plant in Huntington, West Virginia, may have had an increased risk of death from cancer of the respiratory organs. Between 1922 and 1948, nickel-copper sulfide matte, including nickel subsulfide, was oxidized in calciner furnaces similar to those used at Clydach, Wales, and at Port Colborne and Copper Cliff, Ontario [41]. Three deaths from nasal cancer, those of a bricklayer, a laborer [47], and a worker with no apparent exposure to dusts from the calciner furnaces [41], have been identified in these workers. The risk of death from cancer of the respiratory organs was slightly elevated but not statistically significant in retired hourly workers (O/E ratio 1.57) [47], although it was not elevated in all workers (O/E ratio 0.97) [41]. Limited area samples in the calciner sheds contained 5-15 mg of nickel/cu m near the furnace and 20-350 mg/cu m near the grinders. In the melt shop, area samples contained 5-150 mg of total dust/cu m, probably largely nickel [41]. These studies [41,47] suggest that Huntington nickel alloy plant workers may have had slightly elevated risks of developing nasal and lung cancer. It cannot be determined from these preliminary studies, however, if nickel alloy plant workers other than those who were exposed to dust and fume from calciner furnaces would also have an increased risk of developing cancer of the respiratory organs.

Three case reports suggest that exposure to airborne nickel in occupations other than those in nickel refineries may also be associated with the development of cancer of the respiratory organs [62-64]. A case of lung cancer was reported in a 36-year-old man engaged in grinding and polishing nickel-plated material [63], a reticulosarcoma of the nasal fossa

was reported in a 59-year-old woman who electroplated cutlery with nickel [62], and a squamous-cell carcinoma of the nasal cavity was recently reported in a 35-year-old man employed in a nickel stripping operation [64].

On the basis of epidemiologic studies, it appears that the risk of developing cancer of the respiratory organs in nickel refinery workers is related to the extent of exposure to airborne dust, mist, or fume containing nickel. Based on occupational histories of workers who developed lung or nasal cancer, it appears that nickel workers who developed cancer of the respiratory organs were exposed to a mixture of airborne nickel compounds, including nickel subsulfide; nickel oxide; nickel metal; and to nickel salts, such as nickel sulfate, nickel chloride, and nickel carbonate. Exposure to airborne coke, arsenic, or other trace elements or compounds may contribute to the increased risk of developing lung cancer in nickel workers. However, exposure to coke, arsenic, or other trace elements has not been associated with the development of cancer of the nose [75].

The carcinogenic potential of individual nickel compounds has been tested in experimental animals. The experimental data are summarized in Table III-16 at the end of this chapter. Wistar rats did not develop lung tumors over a 15-month observation period after an intratracheal injection of 5 mg of nickel subsulfide [77]. However, rats developed benign and malignant tumors after about 80 weeks of exposure to nickel subsulfide inhaled at a concentration of 1 mg/cu m [78]. Nickel subsulfide has induced a high rate of injection-site tumors in rats [88,89,91] and mice [88]. Kidney carcinomas were found in 45% of rats injected intrarenally

with nickel subsulfide [103]. Hamsters did not have a high rate of injection-site tumors after im injection of nickel subsulfide [95].

Inhalation of nickel powder produced no lung tumors in rats or mice and only a single tumor in guinea pigs [82]. Kim et al [81] found 3 lung tumors in 60 rats exposed to nickel powder by inhalation; 1 lung tumor was observed in 55 control animals. Rats and hamsters did not develop lung tumors when exposed to nickel powder by intratracheal instillation [83]. Lesions suggestive of potential tumor development were noted in several studies [82,83], but these studies did not show that elemental powdered nickel induced malignant tumors under the studied conditions. Nickel powder has also been injected by many other parenteral routes. Rats developed tumors at the site of im injection [99], intrafemoral implantation [96,97], and intrapleural injection [97,105]. Intrarenal injection of nickel did not produce kidney carcinomas in rats [103]. A tumor developed in 1 of 6 rabbits at the site of intrafemoral implantation, but no tumors developed in 10 rabbits exposed to nickel powder by iv administration. No injection-site tumors were induced in mice by iv, im, or intrapleural injections.

Hamsters did not develop lung tumors during lifespan inhalation exposure to nickel oxide; however, two osteosarcomas and one rhabdomyosarcoma were reported [80]. Intramuscular injection of nickel oxide produced injection-site tumors in rats and mice [88]. Rats also developed tumors at the site of nickel oxide implants [101].

Other nickel compounds have also been tested. Nickel carbonate, nickel fluoride, nickel hydroxide, and nickel-iron matte have induced injection-site tumors [94,101,102]. Nickel acetate reportedly induced

tumors at the site of injection [99,101] and also lung adenomas [104]; nickel sulfate [100,101], nickel chloride [101], and nickel ammonium sulfate [101] did not produce injection-site tumors. Nickel-monosulfide injections did not cause kidney tumors in rats [103].

The experimental studies of the carcinogenicity of nickel and its compounds in animals have not been particularly helpful in determining whether or not all forms of nickel may cause tumors. The small nodules, so-called "microcarcinomas," found by Hueper [82] in rats exposed to nickel dust lend support to the conclusion that elemental nickel can cause tumors when particle sizes are small enough for the dust to be airborne; in this case, most particles were smaller than 4 μm. Many other experimental studies in rodents have been less helpful, because of inadequate experimental design, such as using exposure and observation periods that are too short, or perhaps because of the insensitivity of many rodent species or strains to the induction of tumors in the respiratory tract.

Injection-site sarcomas have been induced in rodents by many nickel compounds, but the applicability of these studies to a recommendation for workplace exposure to inorganic nickel is questionable. Injection-site tumors have been induced by compounds that have not caused cancer in animals by other routes of exposure, but it is not clear whether this lack of association between the tumor-producing potential of some compounds by implantation with this potential by other routes of exposure also applies to metals and metal compounds. These data, however, justify recommending precautions to keep nickel from getting into open wounds.

The 1976 edition of the Registry of Toxic Effects of Chemical Substances, published by NIOSH, lists nickel refinery dust as carcinogenic

in humans. Several other nickel compounds are listed as suspected carcinogens in animals. This information was obtained from an IARC monograph on nickel [1]. These studies, together with additional investigations of the carcinogenicity of inorganic nickel, have been reviewed in this chapter.

(b) Genetic Effects

In tests on a variety of microorganisms, eg, viruses [111], bacteria [108], and yeast cells [112], nickel salts gave no evidence of mutagenic activity. Tests on pea and bean root tips have suggested that nickel interferes with mitosis [114-116]. The positive results on plants may have little relevance to safety evaluations for human beings. No reports of mutagenicity tests for nickel in animals were found.

(c) Teratogenicity and Other Effects on Reproduction

Nickel acetate administered iv in doses of 2-25 mg/kg on the 8th day of gestation produced undescribed abnormalities in hamsters [117]. No findings for control animals were reported. Chick embryos did not show abnormal morphologic development when nickel chloride was injected into the egg [119], but nickel chloride solutions interfered with sea urchin egg development [120]. In a recent abstract, Sunderman et al [118] reported fetal toxicity following im injection of nickel chloride into Fischer rats at doses of 12 and 16 mg of nickel/kg. No fetal malformations were noted. Administration of nickel acetate at a dose of 1 mg/kg/day for 15 days reportedly did not affect the reproductive process of male guinea pigs [126], but the study was not reported in sufficient detail.

Nickel salts produced testicular damage in rats and in mice given oral [123], subcutaneous [122], and intratesticular [125] doses of 10-25

mg/kg. In one study, the effect was reversible [122]. In a three-generation study in rats, 5 ppm of nickel in the drinking water, which corresponded to an ingested nickel dose of about 37.6 μg/d for an adult rat [84], had adverse effects on reproduction [121]. Because of the lack of detail in this report, which summarized experimental results on seven metals, it is difficult to determine which parts of the reproductive process were affected. A decrease in the ratio of males to females in the litters and an increase in the number of runts suggests that an effect on gestation, perhaps on transplacental toxicity, occurred. The limited data presented do not clearly indicate whether there were effects on postnatal viability, such as those that might result from an effect on lactation. Studies that are more definitive than this report [121] are needed before these effects might be considered in a standard for workplace exposure to inorganic nickel.

No case reports or epidemiologic studies have been found which address the subject of reproductive or teratogenic effects in humans exposed to inorganic nickel. Single studies on several animal species suggest that some animals are susceptible to reproductive and possibly to teratogenic effects when given nickel by various routes of administration. Research is needed, including experimental animal studies and human epidemiologic studies, to determine whether nickel affects human reproduction.

TABLE III-15

LD50 DATA FOR NICKEL

Compound	Species	Sex	Route of Exposure	LD50 (mg/kg)	Time of Death	Reference
Nickel acetate	Mouse	M	Oral	410	24-72 hr	127
"	"	F	"	420	"	127
"	"	M	ip	32	6-48 hr	127
"	"	F	"	32	"	127
"	Rat	M	Oral	360	24-72 hr	127
"	"	F	"	350	"	127
"	"	M	ip	23	6-48 hr	127
"	"	F	"	23	"	127
Nickel chloride	Mouse	-	"	26	-	170
"	Rat	F	"	11	1 wk	137
"	"	F	"	14	-	171
"	"	F	im	51	-	171
"	"	M	Oral	232	Several d	128
"	"	F	"	285	"	128
Nickel chloride hexahydrate	Mouse	-	ip	48	-	170
Nickel sulfate	"	-	"	38	10 d	172
"	"	M	"	21	30th d	173

TABLE III-15 (CONTINUED)

LD50 DATA FOR NICKEL

Compound	Species	Sex	Route of Exposure	LD50 (mg/kg)	Time of Death	Reference
Nickel perchlorate heptahydrate	Mouse	M	ip	100	4 min-12 hr	174
Nickel oxide (55.9%) and sulfide (20.9%) dusts	Rat	-	it*	110	Over 3 d	136
"	"	-	ip	690	"	136
"	Mouse	-	"	744	"	136

*Intratracheal administration

TABLE III-16

TUMORIGENICITY OF NICKEL COMPOUNDS

Compound	Species	Route of Exposure	Dose (mg)	Dosage Schedule	% Tumors*	Reference
Nickel subsulfide	Rat	Inhal	1.0/cu m	78-80 wk	14	78
				Controls	<1	
"	"	Tracheal	5.0/rat		0	77
"	"	im	20.0/site		80	88
"	Mouse	"	5.0/site		53	88
"	"	"	"		33	88
"	Rat	Implant	250.0/disc	0-32 d	0	89
"	"	"	"	64 d	27	89
"	"	"	"	128 d	73	89
"	"	"	"	256 d	73	89
"	"	im	0.6/rat		27	91
	"	"	1.2/rat		73	91
	"	"	2.5/rat		87	91
	"	"	5.0/rat		100	91
	"	"	10.0/rat		100	92
				Controls	0	92
"	"	"	10.0/rat		100	93
"	"	"	"		52	93
"	"	Renal	5.0/kidney		45	103
Nickel oxide	Hamster	Tracheal	4.0/rat	30 wk	0	79
	"	Inhal	53.2/cu m	Lifespan	3	80
	Rat	im	20.0/site		41	88
	Mouse	"	5.0/site		35	88
	"	"	"		23	88

TABLE III-16 (CONTINUED)

TUMORIGENICITY OF NICKEL COMPOUNDS

Compound	Species	Route of Exposure	Dose (mg)	Dosage Schedule	% Tumors*	Reference
Nickel oxide	Rat	Implant	7.0/rat	3 implants	3	101
Nickel sulfate	"	"	"	"	0	101
"	"	im	5.0/site		0	88
Nickel acetate	"	Implant	7.0/rat	3 implants	3	101
	"	im	35.0/kg		22	99
		ip	360.0/kg	3x/wk x 8 wk controls	1.26** 0.42**	104
Nickel powder	"	Inhal	2.9/cu m	Up to 26 mon Controls	5 2	81
"	Guinea pig	"	15.0/cu m	Up to 15 mon	2	82
"	Rat	"	"	"	0	82
	"	Tracheal	4.0/rat	Repeated at 12 mon	6	83
"	Hamster	im	5.0/hamster	5 monthly	4	95
"	Rat	"	5.0/rat	"	76	95
"	"	"	28.0/rat	Killed at 7 mon	10	100
"	"	"	50.0/rat		66	99
"	"	"	3.6/rat		0	94
"	"	"	14.4/rat		20	94

TABLE III-16 (CONTINUED)

TUMORIGENICITY OF NICKEL COMPOUNDS

Compound	Species	Route of Exposure	Dose (mg)	Dosage Schedule	% Tumors*	Reference
Nickel powder	Rat	Renal	5.0/kidney	Both poles	0	103
"	"	Intra-pleural	5.0/rat	5 monthly injections	20	105
"	"	"	50.0/rat	"	16	97
"	"	Femoral	-		27	96
"	Rabbit	"	-		17	96
"	Mouse	iv	-		0	96
"	Rat	"	-	6 weekly injections	33	96
"	Rabbit	"	-	"	0	96
"	Mouse	im	-		0	96
"	"	Intra-pleural	-		0	96
"	Rat	im	28.3/rat		100	98
Nickel acetate	Mouse	Oral	5.0/liter	Lifespan	17	86
	"			Controls	22	
	"		"	Lifespan	14	85
	"			Controls	32	
Nickel carbonate	Rat	Implant	7.0/rat	3 implants	30	101
Nickel monosulfide	"	Renal	5.0/kidney	Both poles	0	103
	"	im	5.6/rat		0	94
	"	"	22.4/rat		0	94

*Percent of rats with tumors related to treatment; see text for details
**Data given in tumors/rat