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Epidemiological and Experimental Aspects of Metal Carcinogenesis: Physicochemical Properties, Kinetics, and the Active Species

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The carcinogenic properties of selected metals and their compounds are reviewed to provide a useful reference for existing knowledge on relationships between physical and chemical forms, kinetics and carcinogenic potential and between epidemiology, bioassays, and short-term tests. Extensive consideration is given to arsenic, beryllium, cadmium, chromium, lead, and nickel. Other metals such as antimony, cobalt, copper, iron, manganese, selenium, and zinc are discussed briefly.

Introduction

It is more than 100 years since the first report of the possible carcinogenic activity of arsenic appeared. In the intervening period, more metals have been identified as carcinogenic and research has expanded beyond epidemiology. Reviews (*1–5*) usually accept that *a*) the carcinogenic activity of arsenic, chromium, and nickel has been proved by epidemiological studies; *b*) the evidence for the human carcinogenicity of beryllium is inadequate or limited; and *c*) at least in some form or in some experimental conditions many more metals are able to induce tumors or to interact with genetic material.

Although epidemiology supplies the strongest evidence for an association between exposure and carcinogenicity, it has its own limitations. The first source of uncertainty is the lack of information on exposure over a long period of time. Even follow-up epidemiological studies, which incorporate several criteria for the analysis of the cause-and-effect relationship, use semiquantitative approximations and back extrapolations for exposure classification. The distribution of the metal between its different chemical and physical forms, which may have different carcinogenic potential, gives a new dimension to this uncertainty. The second source of uncertainty is confounding variables like age, socioeconomic status, smoking, and co-exposure to other carcinogens at work. A third uncertainty may derive from the long latent period between first exposure and the onset of cancer. The latent period may be longer than the time between the onset of exposure and the cut-off time of the study. The fourth source is the insensitivity of epidemiological studies to detect low risk. The requirements for the epidemiological study of carcinogenicity have been discussed by Doll (*6*) and errors found in several cohort studies are tabulated by the U.S. Environmental Protection Agency (EPA) (*7*).

The selection of experimental conditions to fit the problem makes animal experiments an invaluable tool for the identification of carcinogens. Animal experiments can determine whether a metal in a certain form, dose, and route of administration can induce cancer. The difficulty arises when none, or only some, of these conditions are relevant to human exposure. Thus, the relevance of injection-site tumors, developed after the subcutaneous or intramuscular injection of huge doses of insoluble or hardly soluble metalic powders or compounds, is questionable. It is not without interest that acute inflammatory reactions following intratracheal instillation or inhalation exposure are frequently recorded, but injection site ulceration by definitely ulcerogenic doses (e.g., of cadmium) has been seemingly ignored.

Research on transport through cell membranes (particles by pinocytosis, solutes by diffusion or active transport), metabolic transformation including valency changes and solubility in extracellular and intracellular fluids has helped to give reasonably good ideas about the delivery of carcinogenic metals to the target. *In vitro* tests have helped to identify the active form. Thus, kinetic studies and *in vitro* assays not only supplement, but help to interpret, epidemiological and experimental data. They also confirmed the validity of old-fashioned toxicological principles: There is no effect without contact with the target, as the carcinogenic target (in most cases, genetic material) is within cells, the metal carcinogen must pass through the cell membrane; there can be no primary biochemical reaction with the target without the metal carcinogen being in a reactive form.

However, in spite of the considerable progress made toward the identification of metal carcinogens, the mechanism by which metals exert their carcinogenic effects remain enigmatic for the following reasons: *a*) different *in vitro* evaluation systems (e.g., bacterial cells versus mammalian cells) frequently give contra-

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dictory results (*8*); *b*) many experimental conditions (e.g., intramuscular administration and huge single dose) are not suitable for the identification of crucial steps in a multistage process; and *c*) as carcinogenic metals so far identified have no chemical or biochemical characteristics that cannot be found in some other metals (*9,10*), they are unlikely to act through a common mechanism (like an important group of organic chemicals acts through the alkylation of DNA).

The uncertainties concerning the mechanism of metal carcinogenesis were reviewed by Furst (*10*), who concluded that metal carcinogenesis does not follow the fundamental mechanisms of organic carcinogens and the carcinogenic potential of metals cannot be predicted from the results of short-term tests. The results of *in vitro* tests are used only as guides for the identification of the active species. In agreement with this view, this review does not attempt to make any mechanistic generalizations, but it surveys data on the chemical forms of metals at exposure; the effect of the chemical form at exposure on kinetics and carcinogenicity; and the active form in genotoxic reactions.

The underlying idea behind the inclusion of kinetic considerations after epidemiology and animal experiments is to bridge information on the influence of chemical forms at exposure on carcinogenicity and on activity in short-term tests.

Arsenic

Epidemiology

Though arsenic was the first metal identified as a carcinogen, strong epidemiological evidence in support of the role of arsenic in the induction of skin and lung cancer is relatively new (*11,12*).

Skin carcinomas caused by arsenic are of three types: basal cell carcinoma, squamous cell carcinoma, and Bowen's disease (epidermal squamous cell carcinoma). They are the result of prolonged ingestion of inorganic arsenic in medicine or drinking water. Case reports suggest that occasionally skin cancer is accompanied by cancer in internal organs (e.g., liver cancer, especially hemangiosarcoma), but this association has not been confirmed by epidemiological studies (*13*).

The prolonged oral treatment of chronic dermatoses with diluted Fowler's solution raised the prevalence of skin cancer above 20% in patients whose total dose exceeded 6 g (*1*). Fowler's solution contained 3.8 g As/L in the form of arsenic trioxide. In Tiawan, a survey of more than 40,000 inhabitants established a positive dose–response relationship between arsenic in drinking water (As concentration up to 1.8 mg/L) and skin cancer. Excess morbidity from skin cancer showed a noticeable increase when total As dose exceeded 10 g. A linear, nonthreshold extrapolation indicated that the lifetime risk of skin cancer from the daily oral intake of 1 mg inorganic arsenic is 12.5% (*12*). The multistage-Weibull/time–dose–response model applied for the same data associated 1.3×10^{-3} lifetime risk with the ingestion of 1 μg As/kg/day (*14*). According to the latest study from the southwestern coast of Taiwan, arsenic in drinking water increased the age-standardized mortality ratios (SMRs) of skin cancer deaths of males and females as follows: < 0.3 mg As/L of drinking water by 1.2- and 3.2-fold; 0.3–0.59 As/L by 2.02- and 2.13-fold; 0.59 < ng As/L by 3.42- and 4.32-fold (*15*).

Most of the cancer mortality surveys for occupational groups are from copper smelters. The mortality experience of 8047 white smelter workers (U.S.) exposed to arsenic trioxide indicated a 3-fold increase in respiratory cancer. Based on urinary arsenic concentrations, exposure was graded as heavy, medium, or light. The SMR was as high as 8-fold among employees who had been heavily exposed for more than 15 years. However, as exposure to SO_2 varied concomitantly with exposure to arsenic, cancer mortality and exposure to SO_2 also showed a significant positive correlation (*16*). When the effect of one contaminant was adjusted for the possible confounding effect of the other agent, the relationship of excess carcinogenic risk with arsenic level remained unchanged, but with SO_2 level it disappeared. Owing to the inverse relationship between age at initial employment and employment years, age at initial employment correlated inversely with the SMRs of cancer deaths. Nevertheless, the risk of contracting cancer within a fixed time is increased with age at initial employment (*17*).

In another study, the mortality experience of 2801 smelter workers employed during the period of 1940 to 1964 for at least 1 year was followed through 1976. There were 100 deaths due to lung cancer during the follow-up period. For the purpose of cancer risk assessment, urinary arsenic concentration was a better index than atmospheric concentration (*18,19*). It has been also suggested that the time-weighted exposure is a more consistently reliable predictor of respiratary cancer risk than cumulative exposure (*20*).

A case–referent study from a Swedish copper smelter at Rönnskar found significantly increased lung cancer mortality in the group of copper smelter workers who had been consistently exposed to more than 0.5 mg As/m³ (*21*). Work in the roaster department was associated with the highest risk of cancer, especially lung cancer (*22*). Two follow-up studies used the same cohort of workers employed for at least 3 months from 1928 to 1967. In one study the cohort was restricted to 3710 workers who were alive in 1958 when the Swedish Cancer Register was opened for the registration of all newly diagnosed cases of cancer. Between 1958 and 1982, 461 cancers were registered in the cohort. The 30% excess of cancer incidence over the general population was mainly caused by lung cancer. Thus, compared with the general population of Sweden, the SMR for cancer incidence at all sites was 124 and for lung cancer was 232. SMRs relative to the county population were 133 and 355, respectively. Both the 5 years' moving average of cancer incidence and mortality declined from 1975–1979 to 1980–1984 by about 50% (*23*). In the second study, a positive dose–reponse relationship was found between cumulative arsenic exposure (calculated from atmospheric concentration) and lung cancer mortality. Risk increased with the average intensity, but not the duration, of arsenic exposure. There was no dose–response relationship between exposure to SO_2 and lung cancer (*24*).

In a tin mining cooperative in China, a case–control study compared the exposures to arsenic, radon, and tobacco of 107 employees with lung cancer with the exposures of equal numbers of age-matched tin miners without lung cancer. Exposure to arsenic was categorized with the average exposure in milligrams per cubic meter multiplied by the months of exposure. The study indicated that the risk of lung cancer increased directly with exposure to arsenic, depended more on the duration than on the intensity of arsenic exposure, and appeared to be independent of exposure to tobacco. The high correlation between arsenic and

radon exposure made assessment to each agent alone problematic. However, at the lowest category of radon exposure the relative risk in the low, middle, and high arsenic exposure groups was 1, 2.7, and 5.4, respectively. At the lowest arsenic exposure level, the relative risk of lung cancer was 1, 13.8, and 2.2 in the low, middle, and high radon exposure groups, respectively (*25*).

Two studies (*26,27*) and the update of the first study (*28*) recorded a 3-fold excess of respiratory cancer in employees involved in the manufacture of arsenic-containing insecticides. No excess deaths from cancer were detected among orchard workers in the United States (*12,29*). Though the use of arsenic insecticides by wine growers was abolished in Germany soon after the World War II, in the period of 1960 to 1976, one pathology department diagnosed lung cancer in 108 (66%) of the 163 deceased wine growers with a history of chronic arsenic intoxication. During the same period, the trade association of wine growers had records on 417 deaths. Melanosis, hyperkeratosis, or multiple skin carcinomas were found in 250 cases. The frequency of lung carcinomas was 65.5% when signs of chronic arsenic poisoning were present and 45.9% when they were absent. The concomitant presence of skin and lung carcinomas in wine growers is in agreement with inhalation exposure during spraying and oral exposure to arsenic through the consumption of *Haustrunk* made from the aqueous infusion of pressed grapes. Long fermentation assured an efficient removal of arsenic from grape skins and high consumption (0.7–3.0 L daily) resulted in high arsenic intakes at the time of work in vineyards (*30*).

According to occupational health surveys, increased cancer risk is regularly associated with exposure resulting in lesions of skin and mucous membranes (*13,31,32*). The lack of any similar observation on heavy consumers of seafood and the significantly lower toxicity of organoarsenicals in seafood (*33*) suggest that arsenobetaine is unlikely to be a human carcinogen. Epidemiological studies do not provide evidence on differences in the carcinogenic potential of inorganic arsenicals. It is possible that the lower solubility of arsenic trioxide versus pentoxide may favor longer residence time and carcinogenicity in the respiratory pathway. There are no data on the carcinogenicity of arsine (AsH_3). Exposure to arsine is occasional, and the high acute toxicity of arsine limits the possibility of carcinogenic exposure. Thus, 0.5 mg As/m^3 increased the incidence of cancer after long-term occupational exposure to inorganic arsenic salts (*21*), while only a few hours of exposure to the same concentration of arsine was severely toxic (*13*).

Certain epidemiological observations suggest that arsenic may act as a cancer promoter rather than as an initiator (*34*). These observations indicate that *a*) though cancer usually develops after 20 or more years of exposure, it can develop within 10 years; *b*) effects of exposure disappear after the termination of exposure with time (*18*); and *c*) older persons are more sensitive to the carcinogenic effect of arsenic than younger ones (*17*). The decline in the appearance of new cases after the termination of exposure is in agreement with the decline of arsenic body burden to background level in heavily exposed German wine growers who died 10 to 15 years after exposure (*30*).

The interaction between smoking and arsenic exposure has been the subject of two studies. From the cohort of copper smelter workers studied by Lee and Fraumeni (*16*), 150 men were randomly selected from the heavy exposure group and 150 from the rest of the cohort. Reliable smoking data were obtained from 86% of the sample. Between 1932 and 1977, SMRs for lung cancer were slightly higher for cigarette smokers than for nonsmokers in both groups (*35*). Among Swedish copper smelter workers the combined effects of smoking and arsenic exposure on lung cancer were more than additive (*31,36*).

Animal Experiments

An IARC Working Gr oup (*11*) and a WHO Task Group (*12*) surveyed the literature published before 1980 and found no definite evidence for the carcinogenic activity of inorganic and organic arsenic compounds in experimental animals. The majority of the studies were negative, and, owing to some weaknesses in design (too small numbers, mixed exposure, inadequate controls), the positive studies were judged inconclusive. A recurrent finding of most of the studies published after 1980 is the increased frequency of benign adenomas.

A combination of perinatal and postnatal treatment of mice with trivalent arsenic (0.5 mg As/kg, SC) increased the incidence of lung adenomas from the high background level of 18 to 63% (*37*). The repeated intraperitoneal administration of arsenite did not increase the incidence of lung adenomas in strain A mice (*38*). In 8-week-old Syrian hamsters the weekly intratracheal instillation of As_2O_3 for 15 weeks produced 3 lung adenomas in 10 animals given the total dose of 5.25 mg arsenic, and 2 lung adenomas in 20 hamsters dosed with 3.75 mg/kg. Neither the treated nor the control hamsters developed malignancies (*39*). When the experiment was repeated with the smaller dose, 3 of 47 (6.4%) hamsters developed respiratory carcinomas (larynx, trachea, bronchus, or lung) and 21 (44.6%) other respiratory lesions (adenomas, adenomatoid lesions, and papillomas). The corresponding numbers were 0 and 7 (13.2%) in 53 controls. Combined treatment with arsenic and benzo[*a*]pyrene did not have a synergistic effect (*40*). The weekly installation of 3.0 g As/kg as arsenic trisulfide or calcium arsenate into the trachea for 115 weeks induced 1 adenoma in 28 (3.6%) and 4 in 35 hamsters (11.4%), respectively. No adenomas were seen in 26 controls. The incidences of adenomatoid lesions were 12 (3.6%), 10 (28.6%), and 0, respectively. There were no malignant lung tumors in any of the groups (*41*). Arsenic trioxide, arsenic trisulfide, and calcium arsenate, given to hamsters at the same dose for 2 or 4 weeks, caused toxic local reactions manifested by hyperplastic and metaplastic changes in the epithelium of trachea and bronchi. In calcium arsenate-treated hamsters, there were also signs of proliferation, desquamation, and degeneration of the bronchiolar epithelium with infiltration of macrophages and lymphocytes (*42*).

These experiments indicate that arsenic is tumorigenic and that the tumorigenic intratracheal dose provokes an acute toxic reaction in the lung. The incidence of respiratory cancer in one study was far below the level of significance, while the statistical power of other studies was too low to allow the detection of low incidences of cancer. As far as tumorigenic effect is concerned, the three experiments with identical administration and dosing show that arsenic trioxide [As (III)] and calcium arsenate [As(V)] were more potent than arsenic trisulfide [As(III)] and probably arsenic trioxide was more potent than calcium arsenate. As the order of water solubility is, $As_2O_3 > Ca_3(AsO_4)_2 > As_2S_3$, and the order of retention in lung after intracheal instillation is $Ca_3(AsO_4)_2 >$

$As_2S_3 > As_2O_3$, tumorigenic potential does not seem to correlate either with solubility or pulmonary deposition.

Kinetic Considerations

Solubility is only one factor in pulmonary deposition after inhalation exposure or intrachaeal instillation. Pershagen and Björklund (*42*) suggested that higher wetting capacity of intratracheally instilled particles contributed to the increased pulmonary concentration of calcium arsenate over the concentration of arsenic trioxide or the hardly soluble arsenic trisulfide. Wetting promotes transport to the alveolar region, where clearance is slower than from the bronchial region and where clearance does not increase proportionately with deposition.

About 90% of dissolved arsenic and 30% of suspended arsenic trioxide or trisulfide and lead arsenate is absorbed from the gastrointestinal tract (*43*). Studies on human volunteers indicate that ingested arsenobetaine is nearly completely absorbed from the gastrointestinal tract and most of the absorbed dose is excreted in urine (*44*).

The following summary on the kinetics of absorbed arsenic is compiled from reviews from Vahter (*43*) and Marcus and Rispin (*45*). Arsenic absorbed from the gastrointestinal tract has a shorter half-life than inhaled arsenic because it is transported through the portal circulation directly to the liver, the main site of arsenic biotransformation. Clearance of arsenic can be affected by two toxic reactions: excess As(III) inhibits its own methylation to dimethylarsenic acid and the pulmonary clearance of particles by alveolar macrophages.

Studies on rabbits exposed to radiolabeled arsenate have shown that dissolved arsenate is rapidly reduced in blood to As(III), though some arsenate is excreted unchanged in urine. With time there is a change in the form of arsenic in urine: the percentage of inorganic arsenic declines, and the excretion of methylated forms increases. In addition to dimethylarsinic acid, which is the major component, monomethylarsonic acid is present for a shorter period at a lower concentration. No demethylation takes place, and trimethylation or the reduction of dimethylarsonic acid to volatile dimethylarsine is not significant. The rapid excretion and low toxicity of dimethylarsinic acid (its LD_{50} is 350-fold higher than the LD_{50} of arsenic trioxide) is explained by its low affinity for tissues. After the ingestion of soluble inorganic arsenic salts, the clearence half-time in human subjects is about 5 days, while after the ingestion of dimethylarsenic acid or arsenic in fish, the clearance half-time is about 2 to 3 days. When the inhaled arsenic is in sparingly soluble form, reduction and methylation is slow, and clearance is delayed. These factors are most likely responsible for high concentrations of arsenic in the lungs of smelter workers several years after the cessation of exposure.

The conversion of As(V) to As(III) may be one reason why animal carcinogenicity studies do not differentiate between the carcinogenic potential of these two forms, though toxicity studies indicate that As(III) is more toxic and more reactive than As(V). As(III) reacts with sulfhydryl groups, and monosubstituted arsenite forms stable bonds with two vicinal sulfhydryl groups (e.g., in lipoic acid dehydrogenase). One possible consequence of this reaction is the inhibition DNA repair enzymes. Though the oxyanion of As(V) can replace phosphate in substrates and can inhibit phosphorylation, the arsenate ester bond is easily hydrolyzed. After treatment with As(V), most of the arsenic in the nuclei of cells is in the form of As(III) (*46*). These metabolic considerations indicate that As(III) is more likely to participate in the carcinogenic process than is As(V). It has been also suggested that arsenic intake must exceed the methylating capacity of the body before any skin lesions (hyperkeratosis or skin cancer) are produced (*43*).

Short-Term Tests

Arsenic compounds were consistently inactive in the Ames Salmonella test with or without the addition of S-9 mix (*4*). Arsenite and arsenate were also negative in assays with *S. typhimurium* and with the tryptophan auxotrophic *E. coli* WP2 strain (*33,47,48*). Arsenite did not induce λ prophage in *E. coli* WP2s (*49*). There are conflicting reports on the mutagenicity of arsenic in the *B. subtilis rec* assay, a test which is claimed by some to be more sensitive for screening metal carcinogens than Salmonella or *E. coli* (*34*). In yeast, both arsenite and arsenate induced gene conversion (*50*).

In mammalian cell (gene) mutation assays, arsenate and arsenite were negative or weakly mutatagenic. Thus, As(III) was weakly mutagenic at the thymidine kinase locus in mouse lymphoma cells (*51*), and it did not induce ouabain-resistant mutants in Chinese hamster V79 cells (*49*). Arsenic was not mutagenic in the dominant lethal test in mice, though it increased the mutagenicity of Tepa (tris-1-aziridyl-phosphine oxide) (*52*).

Pentavalent arsenic induced morphological transformation in Syrian hamster embryo cells (*53*), and trivalent enhanced viral transformation in the same system (*54*). In C3H/10½Cl 8 mouse embryo cells, As(III), but not As(V), induced morphological transformations but not ouabain resistance. This indicated that transformation was not caused by base substitution mutation. Both As(III) and As(V) induced stable anchorage independence in diploid human fibroblasts (*55*). At low concentrations, arsenic enhanced the colony-forming efficiency of Syrian hamster embryo cells followed by dose-dependent inhibition at higher concentrations. As(III) was about 15 times more potent than As(V). This difference in potency was seen also in the induction of morphological transformation in Syrian hamster embryo cells and the induction of methotrexate resistance in mouse 3T6 cells (*56*). Cellular uptake, cytotoxicity, and morphological neoplastic transformation in the mouse embryo cell line BALB/3Y3 clone A31-1-1 were 4-fold higher for As(III) than for As(V), though As(V) was converted intracellularly to As(III). The foci transformed by As gave rise to tumorigenic cell lines (carcinomas in nude mice) (*57*).

Both As(III) and As(V) increased polyploidy, chromosomal aberrations and sister chromatid exchange (SCE) in Syrian hamster embryo cells in a concentration-dependent manner, but arsenite was at least 10 times more potent than arsenate (*58*). As(III) was more cytotoxic than As(V) in Don Chinese hamster cells, but the inducibility of SCE was almost identical at their median inhibitory dose (*59*). In cultured human lymphocytes, only arsenite caused SCEs, and arsenite caused significantly more gaps and chromatid and chromosome breaks than did arsenate (*60,61*). In human fibroblasts, more chromosome damage was caused by As(III) than by As(V) (*59*). As excess frequencies of chromosomal aberrations and SCE were observed in the peripheral lymphocytes of exposed human beings, these *in vitro*

Arsenite, but not arsenate, inhibited excision repair in *E. coli* WP2, as shown by enhanced susceptibility to the mutagenic effects of ultraviolet radiation (*47*). Post-treatment with sodium arsenite also potentiated the cytotoxicity, clastogenicity, and mutagenicity of ultraviolet light (*57*) or methyl methansulfonate (*62*) in Chinese hamster ovary cells. While post-treatment with arsenite increased, pretreatment decreased the hypoxanthine-guanine phosphoribosyltransferase mutagenicity of methyl methansulfonate (*62*). Arsenite in relatively low concentrations also potentiated the mutagenicity of *N*-methyl-*N*-nitrosourea at the *hprt* locus of V79 cells (*62*). The interaction with UV, methyl methansulfonate, and *N*-methyl-*N*-nitrosurea indicates that arsenic is a co-mutagen. Arsenic did not affect the fidelity of DNA synthesis in a cell-free *in vitro* test, while Be, Cd, Cr, Ni, and Pb did (*64*).

Because arsenic can be methylated *in vivo*, the genotoxic role of dimethylarsenic has attracted some attention. In the lung tissue of mice, the oral administration of 1.5 g/kg sodium dimethyl-arsenic acid resulted in DNA single-strand breaks which were rapidly repaired. In *in vitro* experiments with lysed and digested nuclei, isolated from control mouse lung, strand-breaks were produced by a reduction product of dimethyl-arsenic acid, dimethylarsine, but not by dimethylarsenic acid (*65*). As the dose given to mice was near to the LD_{50} of dimethylarsenic acid and it delivered 25 times more As(III) than did the oral LD_{50} of arsenic trioxide (*32*), this finding has little or no practical significance for exposure to inorganic arsenic or even to dimethylarsenic acid.

The genotoxicity of inorganic arsenic was extensively reviewed by Jacobson-Kram and Montalbano (*66*), who concluded that the relative potencies of trivalent and pentavalent arsenicals as sulfhydryl reagents are similar to their relative potencies as clastogens and SCE-inducing agents.

Conclusions

Contrary to epidemiological studies, animal experiments have not provided definite evidence for the carcinogenicity of arsenic. Arsenic is not active in gene mutation tests, but it increases chomosomal breaks, sister chromatid exchange, and morphological transformation in mammalian cells. Leonard (*52*) interpreted the positive clastogenic and negative mutagenic effects as an indication of co-carcinogenicity mediated through inhibition of repair. There is some epidemiological evidence to support the view that arsenic more likely acts as a promoter and not an initiator. *In vitro* tests indicate that arsenite is a more potent genotoxic agent than arsenate and arsenate acts only after conversion to arsenite and that the active agent is probably As^{3+}.

Beryllium

Epidemiology

Beryllium can cause skin lesions, acute repiratory inflammation, and progressive interstitial pulmonary granulomatosis, known as chronic beryllium disease. While acute respiratory inflammation, a reversible illness, is mainly the result of high exposure to soluble beryllium salts, chronic berylliosis is the result of prolonged exposure to mostly insoluble beryllium compounds. The collection of case reports registered from 1952 onward in the Beryllium Case Registry at the Massachusetts General Hospital and cohort studies were the basis for several overlapping epidemiological reports on the carcinogenic effect of beryllium. These epidemiological studies have been extensively reviewed (*2,7,67–69*).

These studies showed a consistent pattern of elevated age-standardized observed-to-expected ratios of 1.3 to 1.6 for lung cancer in workers exposed to beryllium for less than 5 years, with the highest ratio in the 3 to 15 month exposure group. Lung cancer showed elevated ratios only among workers who had experienced acute berylliosis, but not in workers with chronic berylliosis. The difference can be explained by the fact that acute berylliosis is always a sign of toxic exposure, while chronic berylliosis is a hypersensitivity reaction. Increased cancer death occurred after 15 years of the onset of Be exposure.

Critics of these epidemiological studies pointed out *a*) the influence of overlapping cases on the convergence of the results of several studies; *b*) the lack of dose–response or length of exposure–incidence correlation; *c*) the lack of consideration for possible confounders including smoking history even when it was available; *d*) manipulative use of input data including unjustifiable low control lung cancer mortality; *e*) inclusion of a lung cancer victim who did not satisfy the condition for inclusion; and *f*) lack of adequate control group selection (*7,68*). A co-author of a report publicly disowned the content because the paper failed to mention that 42 of the 47 lung cancer death cases smoked cigarettes, and 1 of the remaining 5 cases died from causes other than lung cancer. After adjusting for cigarette smoking, the significant elevation of lung cancer mortality disappeared (*7*).

These revelations naturally affected the acceptance of the epidemiological evidence more in the United States than elsewhere. Thus beryllium is considered to be an established carcinogen in the working environment in Sweden and in the Federal Republic of Germany (*2*), while the International Agency for Research on Cancer (IARC) (*11*) classified the human epidemiological evidence as limited, and EPA (*7*) classified it as inadequate.

Animal Experiments

Gardner and Heslington (*70*) observed osteosarcoma in each of nine rabbits injected intravenously with zinc beryllium silicate. Since the publication of this report experiments on the carcinogenic potential of beryllium have covered every possible route of administration and several beryllium species from soluble beryllium compounds to alloys. Several short reviews (*2,4,68*) and a comprehensive evaluation by EPA (*7*) summarize the conclusions of these studies. The review by EPA, which tabulates data separately for each route of administration, is the main source of the following summary.

Experiments demonstrated that the IV injection of zinc beryllium silicate in rabbits, but not in rats and guinea pigs, produces consistently metastasizing osteosarcomas in the long bones. Similar results were obtained with beryllium oxide, beryllium phosphate, and beryllium metal or by injecting beryllium into the medullary cavity of bones. No other route of administration led to the formation of osteosarcomas. Splenectomy increased carcinogenicity. The tumors were chondroblastic, osteoblastic, or fibroblasite (*7*).

The repeated intratracheal instillation of beryllium metal, beryllium oxide, beryllium hydroxide, and beryllium–aluminum alloy with 62% beryllium caused pulmonary carcinomas in rats and monkeys. Alloys with low (4% or less) beryllium concentrations were ineffective. Firing temperature affected the carcinogenic potency of BeO. Cancer incidence was minimal after the instillation of high-fired (1600°C) or medium-fired (1100°C) BeO and high after the instillation of low-fired (500°C) BeO.

Inhalation exposure of rats to beryllium sulfate, beryllium phosphate, beryllium fluoride, zinc beryllium manganese silicate, beryl ore, but not bertrandite ore, were also carcinogenic in rats. Rhesus monkeys were less sensitive, and rabbits, hamsters, and guinea pigs seemed, in most studies, to be resistant. Thus, exposure that gave 100% carcinogenicity in rats was ineffecive in guinea pigs. On a 35 hr week exposure schedule, 10 μg Be/m^3 (as $BeSO_4$) was the threshold for the induction of pulmonary adenocarcinoma in rats (*68*). Most of the malignancies were adenocarcinomas with a predominantly alveolar pattern. A Soviet study reported a dose–dependent increase in the incidence of lung carcinomas in rats exposed to $BeCl_2$ or BeO concentrations ranging from 0.8 to 400 μg Be/m^3 for 1 hr/day, 5 days/week, for 4 months. The lung tumors were squamous cell carcinomas, adenocarcinomas, and undifferentiated carcinomas (*71*).

Neoplasmas were not observed after intracutaneous injection or percutaneous applications and there is no experimental evidence that the oral administration of beryllium is carcinogenic. In the lung adenoma assay, $BeSO_4$ was weakly positive in strain A mice (*72*).

Kinetic Considerations

Beryllium is an amphoteric element and therefore it is able to form positive or negative ions in acidic or basic media, whereas in the buffered environment of tissues, beryllium salts are readily precipitated as colloidal beryllium phosphate/beryllium hydroxide complexes. The precipitation of beryllium salts in blood after IV injection leads to retention mainly in the reticoloendothelial system and bone. The removal of spleen, this important storage organ, increased the carcinogenicity of IV-injected beryllium in bones, most likely by allowing the retention of a higher proportion of the dose in this target (*7,73*).

After ingestion, about 20% of the dose is absorbed from the acidic stomach while the remaining part of the dose is precipitated in the gut to an insoluble form (*68*). Pulmonary clearance is multiphasic; a fast clearance rate is followed by rapidly diminishing elimination rate. After prolonged inhalation exposure to $BeSO_4$, pulmonary clearance half-time was approximately 2 weeks and thereafter diminished rapidly. Though soluble beryllium salts also precipitate out in the respiratory tract, their initial clearance is faster than the clearance of insoluble or sparingly soluble forms (*68*).

Uptake by cells is mainly by endocytosis (the phagocytosis of solids and the pinocytosis of liquid droplets) and clearance is by macrophages and by the mucociliary escalator. When alveolar macrophages are damaged by the engulfed beryllium, lysosomal enzymes are released and clearance is slowed down temporarily. After this initial period of inhibitions phagocytic activity is stimulated by beryllium (*72,74*). After a single 1-hr inhalation exposure of rats to 4 mg Be/m^3 (as $BeSO_4$), the signs of cell deaths and increased phagocytic activity in the bronchoalveolar lavage persisted at least for 1 year (*75*).

The presence of beryllium in lysosomes is only temporary. The acidic milieu of lysosomes is favorable to the dissolution and dissociation of beryllium compounds to ionic beryllium. The cytotoxic potential increases with dissociability and that is why colloidal beryllium hydroxide is more cytotoxic than particulate beryllium phosphate, and more BeO than $BeSO_4$ is required to damage macrophages (*74,76,77*). In surviving cells (e.g., lung tissue) there is a gradual transfer of beryllium to the nucleus, where beryllium is associated mainly with highly phosphorylated nonhistone proteins with consequent interference with the phosphorylation of nuclear proteins and cell division (*72*). As beryllium ions are released from the surface of particles, the conversion of mass to surface concentration (area/volume of medium) resulted in similar expression of cytotoxicity by the different physical forms of the same beryllium compound (*78*).

The damage to macrophages by incorporated beryllium leads to greater infiltration by macrophages and lymphocytes and ultimately to the formation of immunological granuloma—a hypersensitivity reaction. This condition, which was the cause of the highest beryllium-related mortality among exposed workers, was caused mainly by high-fired beryllium oxide, whereas acute pulmonary disease was caused by high exposure to soluble beryllium compounds. Besides the exclusion of high- and and medium-fired beryllium oxides, no similar categorization is possible for the carcinogenic potential of different forms of beryllium. A point of interest is that guinea pigs do not readily respond with tumors to beryllium exposure but show granulomatous dermal hypersensitivity, which can be prevented by inhalation exposure to beryllium sulfate. This seems to indicate that beryllium-induced granulomatous hypersensitivity and carcinogenicity are mutually inhibitory processes (*68,73,74*).

Short-Term Tests

The selection of beryllium species for short-term tests has been dictated not by their importance in human exposure, but by their solubility. Thus, in a table on the activity of beryllium compounds in *in vitro* assays, Baker (*9*) listed one entry for an unspecified form, seven entries for $BeSO_4$, and five for $BeCl_2$.

Beryllium was not mutagenic in plate incorporation assays with Salmonella strains (*7,69,72*). Nevertheless, in the fluctuation assay, $BeSO_4$ was mutagenic to Salmonella TA100 (but negative to other strains) and to *E. coli* (*48*). Both amber and ochre mutations were induced by $BeCl_2$ in the *lacI* gene of *E. coli*, primarily involving base substitution (*79*). $BeSO_4$ was weakly positive in the rec assay with *Bacillus subtilis*, and it was negative in the following: *pol* assay (which measures the growth-inhibiting properties of mutagenic agents in repair proficient and deficient strains) of *E. coli*; host-mediated assay; mitotic recombination assay in yeast; and DNA repair assay in hepatocyte primary culture (*7*).

The induction of 8-azaguanine-resistant mutants by $BeCl_2$ in Chinese hamster V79 cells and by $BeSO_4$ in Chinese hamster ovary (CHO) cells has demonstrated the ability of beryllium to cause gene mutations in cultured mammalian cells (*7*). In

Chinese hamster lung cells, $BeSO_4$ caused no chromatid or chromosomal aberrations (*72*), but it produced chromosomal breaks and SCE in cultured human lymphocytes, Syrian hamster embryo cells (*80*), and CHO cells. Chromosomal damage in CHO cells was more than additive when cells were treated with beryllium sulfate and X-rays (*81*).

$BeCl_2$ decreased the fidelity of DnA synthesis in the presence of poly[d(A-T)] template in a cell-free *in vitro* system, probably by the interaction of Be^{2+} with DNA polymerase (*64*). Similar results were obtained with other systems (*72*).

Conclusions

Animal experiments have supplied sufficient evidence for the carcinogenicity of beryllium and its compounds administered by routes relevant to human exposure. These experiments also demonstrated wide species differences in the carcinogenicity of beryllium, rats being the most sensitive and guinea pigs the most resistant. The inadequacy of epidemiological evidence for the human carcinogenicity of beryllium indicates that human sensitivity must be well below the sensitivity of rats. In the pre-1950 period, the atmospheric beryllium concentration of several workplaces in beryllium production plants was around 1000 $\mu g/m^3$ (*68*), while the median effective concentration of beryllium sulfate for the induction of pulmonary carcinoma in rats (weekly 35-hr exposure for 3 or more months) was 18 μg Be/m^3 (*7*).

The exclusive use of highly soluble beryllium compounds in short-term tests limits extrapolation to other forms, but, in agreement with animal experiments, these assays indicate that dissociation to Be^{2+} is a key step in the carcinogenic process.

Cadmium

Epidemiology

A possible association between exposure to CdO and cancer was first raised in the United Kingdom by the death certificates of eight Ni–Cd battery workers with 14 to 38 years of exposure to cadmium. Five of the eight workers died of cancer, three of them from prostatic cancer (*82*). This finding initiated the epidemiological survey of 248 workers with at least 1 year of exposure. The identification of one additional prostatic cancer case resulted in a 4/0.58 observed/expected (O/E) mortality ratio, while the 0/E ratio was 5.0/4.4 for lung cancer (*83*). The resultant epidemiological studies, from the United Kingdom, United States, and Sweden, covered not only Ni–Cd battery plants but also Cd smelters and the production of Cd compounds and alloys. These studies were the subjects of several reviews (*2,84–87*).

The outcome of these epidemiological studies was controversial. Thus, in 1985 Elinder et al. (*88*) argued that epidemiological studies "support an association between lung cancer and cancer of the prostate and the exposure to cadmium" while Kazantzis and Lam (*89*) argued that the association was not valid for prostatic cancer and inconclusive for lung cancer. These conflicting views reflected disagreement over the judicious use of mortality data (for prostatic cancer) and the role of confounding factors (lung cancer).

According to Doll (*6,90*), when an unexpected finding is observed and other studies are made to check it, the hypothesis-forming data must be excluded from subsequent analysis. In the same nickel-cadmium battery factory, where the first four cases of prostatic cancer occurred, the follow-up of 2559 male nickel–cadmium battery workers found four additional deaths from prostatic cancer during the period of 1946–1981. The new cases with the four old cases gave a 8/6.6 O/E ratio and without the hypothesis-forming cases the SMR declined below below 100 (*91*). The condition of inclusion in this study was start of employment between 1923 and 1975 and at least 1 month of employment. When the cohort was decreased to 458 workers with a minimum employment of 1 year and a job involving high exposure to cadmium (negative active material, assembly, plate making), the O/E ratio for prostatic cancer incidence became 8/1.99 with and 4/1.78 without the hypothesis-generating four cases. The 4/1.78 for new cases was not significant (*92*).

In a Swedish Ni–Cd battery plant, a study found 2/1.2 O/E ratio for prostatic cancer (*93*). The extension of this study resulted in 4/2.4 and 4/2.7 O/E ratios (*94*). In a Swedish cadmium alloy factor four workers died of prostate cancer versus 2.69 expected (*93*).

Less than expected prostatic cancer deaths were found among 331 cadmium-copper alloy workers exposed to a mean level of 70 μg Cd/m^3. In vicinity workers, who had been exposed to only 6 ± 8 (SD) $\mu g/m^3$ Cd, the observed-to-expected ratio of prostatic cancer mortality was 8/3 (*95*). Vicinity workers were engaged in the manufacture of arsenicals and copper and had more than 10 times lower cadmium exposure than cadmiu–copper alloy workers. Based on the low level of exposure and the presence of confounding exposure, it seems clearly unjustified to use, as Elinder et al. did (*94*), the 8/3 O/E ratio of vicinity workers or 9/4.7 ratio of the combined groups of cadmium alloy and vicinity workers to support the etiological role of cadmium.

Armstrong and Kazantzis (*96*) included in their study all major plants in England in which processes involving cadmium had been carried out. The exceptions were the nickel–cadmium battery factory and the copper cadmium alloy plant described above. The mortality experience of 6995 workers born before 1940 and exposed to cadmium for more than 1 year between 1942 and 1970 was followed up to 1979. O/E ratio for prostatic cancer was 23/23.3 in the whole group and 0/2.9 in the "ever high" (3%) and "ever medium" (17%) exposure groups (*96*). A 5-year update found the ratios 30/33.2 for the total population and 0/4.6 for the high and medium exposure groups (*97*).

The first study in a Cd-smelter in the U.S. seemed to confirm the role of cadmium in prostatic cancer. The vital status of 292 cadmium production workers with at least 2 years of employment was recorded from the first day of 1940 to the last day of 1969. Employment years were given only for cases of prostatic cancer death and without stating whether employment was continuous or intermittent, or whether it started before 1926, when the plant operated as an arsenic smelter, or later. There were four prostatic cancer deaths, which occurred 25 to 32 years after first employment, versus 1.15 expected (*98*). An extension of this study to 602 workers with at least 6 months of exposure found no new deaths from prostatic cancer. Moreover, when the classification of the cohort required not only 2 years minimum employment, but at least 6 months of exposure, one prostatic cancer case had to be removed, and the original 4/1.15 O/E ratio (SMR = 347) was reduced to 3/2.2 (*99*).

Thus, epidemiological studies failed to support the role of cadmium in the causation of prostatic cancer. When O/E ratios of last follow-ups are summed up with the O/E of cadmium–copper alloy workers, the result is an insignificant 16/13.8 and not the significant 28/17.2 as calculated by Elinder et al. (*94*). The occupational data are supported by the comparison of latent and fatal prostatic cancers in Japanese living in Hawaii and Japan. Latent prostatic carcinoma is equally common in the two communities, but in spite of significantly higher dietary intake of Cd in Japan, the mortality from cancer of the prostate is higher in Hawaii (*100*).

Contrary to prostatic cancer, epidemiological studies, with the exception of one (*95*), consistently showed increased lung cancer SMRs. The excesses were not always significant and not always increased with exposure.

In the cohort of 2559 male Ni–Cd battery workers, an insignificant increase of prostatic cancer mortality was associated with a slight but significant increase of lung cancer. There were 87 cases of respiratory cancer deaths versus 68 expected (SMR = 128). Exposure to cadmium ranged from 0.6 to 2.8 mg/m^3 before 1950, declined to < 0.5 mg/m^3 after 1950, to < 0.2 mg/m^3 after 1967, and to and below 0.05 mg/m^3 after 1975. Possible confounding factors associated with work were welding with oxyacetylene and exposure to nickel hydroxide (*91*).

In a follow-up, the mortality experience of 3025 workers employed by the same battery plant in the period of 1923 to 1975 was analyzed (*101*). The estimated cadmium exposure (duration of employment) of those who had died in the period of 1946 to 1984 from lung cancer was compared with the exposure of those who survived the same year and started employment at the same age and same year. Among workers first employed in the period of 1923 to 1946, there was some evidence of an association between the risk of dying from lung cancer and duration of employment in high- and moderate-exposure jobs, although the evidence relied heavily on the findings for the single highest exposure (more than 15 years employment) category. Among workers first employed in the period of 1947 to 1975, there was no evidence of such an association (*101*).

In a Swedish Ni–Cd battery factory in a group of 228 workers, 2 died of lung cancer versus 1.35 expected between 1959 and 1975 (*93*). In an extended study, 522 workers were included. There were 8 deaths from lung cancer versus 4.1 expected (SMR = 133) between 1951 and 1983. All had at least 5 years of exposure, and with one exception all died at least 20 years after the onset of exposure. Most of those workers who developed cancer had started to work between 1948 and 1960 when, owing to heavy exposure, 46% of the workers had proteinuria compared with 9% after 1963. Thus, the level of cadmium exposure was 1 mg Cd/m^3 before 1947, about 0.3 mg/m^3 in 1947 to 1962, 0.05 mg/m^3 in 1962 to 1974, and 0.02 mg/m^3 after 1975. Exposure to nickel hydroxide was usually 2 to 10 times higher than exposure to cadmium (*94*).

In the study of Armstrong and Kazantzis (*96*), which included 6995 workers exposed to cadmium for more than 1 year between 1942 and 1970, there was a marginal excess (199 vs. 185.5, SMR = 107) of lung cancer mortality. This excess occurred mainly in the "always low exposure group" and was absent in the "always high exposure" group. A 5-year update of this study found the SMR for lung cancer rising from 107 to the significant (277/240.9) 115 level. SMR was the highest in the very small (12 observed, 6.2 expected) "ever high" exposure group. SMR showed a positive, though not significant, correlation with exposure. Analysis of mortality pattern showed that lung cancer risk increased when employment exceeded 20 years, started before 1940, and the follow-up period was long. Work in the lead–zinc–cadmium smelter, and to a lesser extent in copper–cadmium alloy manufacture increased risk (*97,102*).

A nonferrous smelter was the subject of a more detailed study (*103*). In a cohort of 4393 men, the SMR for lung cancer was 124.5 with 182/146.2 O/E ratio. SMR increased with years of employment, but the increasing risk of lung cancer was not restricted to a particular process or department and could not be accounted for by cadmium. Association between mortality and graded exposure to several environmental contaminants was examined with matched logistic regression. Relative risk did not increase with increasing exposure to cadmium, zinc, SO_2, or dust, but it increased with increasing exposure to arsenic and lead.

In the mortality study of 292 United States cadmium production workers (*98*), there were 12 observed respiratory cancer deaths versus 5.11 expected. Ten of the twelve respiratory cancer deaths occurred at least 15 years after first employment (*94*). An extension of this study to 602 workers with at least 6 months of exposure confirmed increased mortality from respiratory cancer. The respiratory cancer mortality ratio was 3/0.56 for those who were hired before 1926 and 15/7 for those hired later (*99*). Before 1950, in the production departments, exposure to atmospheric Cd was generally slightly greater than 1.0 mg/m^3 with occasional values over 10 mg/m^3. After 1950, exposure gradually decreased to 0.6 mg/m^3 and below 0.2 mg/m^3 in other departments. Based on very low ($\sim$ 1 μg/m^3) arsenic concentrations measured in 1973 in two atmospheric samples from the premeld and one sample from the retort department, the first report (*98*) rejected the role of arsenic as a possible confounding factor. Contrary to these three low values, arsenic concentrations at roasting and calcine furnaces were 300 to 700 μg/m^3 in 1950 and 100 μg/m^3 in 1979 (*99*).

The discrepancy between different epidemiological studies may be attributable to differences in cadmium exposure. Thus, in the United States, cadmium production plant workers were exposed to 100 to 1500 μg Cd/m^3 before 1960 (*95*), while the average exposure of smelter workers studied in the United Kingdom was only 60 μg/m^3 (*93*). In the United Kingdom Ni–Cd battery plant, increased incidence of lung cancer was found only in workers employed before 1947 but not later (*101*). Another explanation is the presence of confounding variables: Thus, in the second report on United States cadmium production workers, it was assumed that arsenic exposure should have resulted in not more than 0.77 lung cancer cases (*99*). However this value was based on the following assumptions: *a*) atmospheric concentrations measured in 1950 or later were representative of concentrations between 1926 and 1950; *b*) respirators were worn according to regulations and the use of respirators decreased exposure by 75%; and *c*) only 30% of the working day was spent in high-exposure areas. These assumptions may have introduced a substantial error in the analysis even without an additional problem caused by the identity of areas (like roasting and calcine furnaces) with high cadmium and arsenic concentrations.

Epidemiological studies on cadmium-exposed workers have not given information on the contribution of smoking to the induction of lung cancer and did not differentiate between the different chemical and physical forms of cadmium. The lack of smoking data is always a cause for concern, but the problem is amplified when the target is the lung and there is a concomitant exposure to arsenic. Thus, in a Swedish copper smelter the age-standardized ratio for death from lung cancer was 3.6 for arsenic-exposed nonsmokers, 4.9 for smokers without arsenic exposure, and 14.6 for arsenic-exposed smokers (*36*). The multiplicative effect of smoking and arsenic exposure on lung cancer death presents a logistic problem for the selection of matching controls.

Animal Experiments

In the first experiments on the carcinogenic potential of cadmium, the route of administration was either intramuscular or subcutaneous. The injection of suspensions of 25 mg finely divided cadmium, cadmium sulfide or oxide regularly gave rise to rhabdomyosarcomas or fibrosarcomas in rats. The tumors were metastatizing and pleomorphic with high yield (*2*). It is well known that the injection of cadmium at this dose level has a necrotic effect at the site of injections and necrosis may have contributed to the development of nonmetastasizing testicular tumors in rats and mice treated subcutaneously with soluble cadmium salts (chloride or sulfate). Thus, the weekly SC injection of 86.5 μg cadmium as $CdSO_4$ for 2 years (approximate total dose = 25 mg/kg) produced no testicular or prostatic changes (*104*). The same cadmium compound given in single IM dose of 3.4 mg Cd/kg regularly led to the development of prostatic adenomas. The same dose given subcutaneously induced testicular instead of prostatic adenomas (*105*). The induction of local sarcomas and testicular tumors was prevented by the administration of zinc acetate (*2*). The interaction of zinc with cadmium is a complex phenomenon. Zinc acetate in a dose of 1 mmole/kg changed the target of SC $CdCl_2$ from the testis to the prostate, but it decreased the incidence of prostatic adenomas from 42% to 25% after the IM administration of cadmium (*105*). Carcinomas in the prostate were produced in rats only rarely when 1.2 mg Cd/kg as $CdCl_2$ was injected into the ventral prostate (*106*).

No carcinogenic responses were observed in seven studies (five on rats and two on mice) as a result of lifetime exposure to cadmium in drinking water or food or by gavage. There is no evidence that cadmium is carcinogenic by the dermal route (*107*).

Inhalation experiments with cadmium aerosols indicated that cadmium is not carcinogenic in the hamster or the mouse. Exposure time was 19 hr/day, 5 days/week, and exposure lasted for 18 months, followed by a 6- to 12-month observation period. Concentrations ranged from 30 to 90 μg Cd/m^3 for $CdCl_2$, 10 to 270 μg/m^3 for CdO, and 30 to 1000 μg/m^3 for CdS. Some groups were exposed 8hr/day. Tumor incidence was low in hamsters and high in mice, but in both species no exposure-related tumor incidences occurred. Thus, the percentage of tumor-bearing mice in the exposed groups varied between 2.6 and 35.6%, while in control groups between 14.6 and 36.9%. Tumor types were not described (*108*).

Rats were exposed to atmospheric cadmium 22 to 23 hr/day, 7 days/week, for 18 months, and surviving rats were observed for a further 13-month period (*109–113*). Carcinogenicity and mortality data are summarized in Table 1. As no sex difference was seen, data for males and females are grouped together. Table 1 indicates that CdO fume was less carcinogenic than CdO dust or $CdCl_2$, $CdSO_4$, or CdS. Solubility did not influence carcinogenicity. Owing to high mortality, the 18-month scheduled exposure period had to be terminated earlier for some groups exposed to 90 or 270 μg Cd/m^3. For continuously exposed rats, the lowest level of exposure that increased mortality was around 50 μg/m^3, and 90 μg/m^3 was lethal to a significant proportion of the exposed group (*109,110*). Exposure to $CdCl_2$ or CdO dust at 30 μg/m^3 Cd concentration induced lung tumors in about 75% of rats, and a high proportion of tumors were malignant. In a separate study, 6 of 40 rats (15.4%) exposed to 12.5 μg Cd/m^3, in the form of $CdCl_2$ developed primary lung carcinomas (*111*).

Gross necropsy of dead animals showed that exposure to cadmium aerosol increased lung weight and enlarged thoracic lymph nodes. Cytometric analysis of bronchoalveolar lavage and pulmonary histology showed that exposure to 30 μg/m^3 caused significant inflammation, progressive interstitial fibrosis, and brochiolo–alveolar hyperplasia (*112*). Adenocarcinoma was the most common lung tumor, followed by adenoma and squamous cell carcinoma (*113*). Exposure to 10 μg Cd/m^3 (as CdO) neither induced tumors nor increased cell debris in bronchoalveolar lavage. Zinc aerosol in a 10 to 1 Zn/Cd ratio prevented the tumorigenicity of CdO in the 30 μg Cd/m^3 exposure group and reduced the tumorigenicity of 90 μg Cd/m^3 (*107*). Treatment with zinc reduced mortality to the control level in both groups (*109*).

When exposure time was reduced to 40 hr per week and the exposure period to 6 months, pulmonary cancer developed only in 2.5% of rats exposed to 270 μg Cd/m^3 as CdS and in 17.5% of rats exposed to 90 μg Cd/m^3 as CdO dust. Mortality was 0 and 2.5%, respectively (*109,110,112*). As daily exposure time and duration of exposure were decreased together, it is impossible to proportion responsibility for the decrease in carcinogenicity or mortality. Nevertheless, the outcome of experiments with standard and reduced exposures suggests that the carcinogenic exposure level just lagged behind the level of lethal exposure and was within the level that caused inflammatory reactions.

In a test of the pulmonary toxicity of continuous exposure to 100 μg Cd/m^3 as CdO, the same research team found definite

Table 1. The mortality and carcinogenicity of continuous exposure (22 hr/day, 7 days/week) to different cadmium compounds in Wistar rats.[a]

Compound	Cd concentration, μg/m^3	Exposure, months	% of rats: 24-Month mortality	With lung tumors	With lung malignancies
Control			7.5	0	0
$CdCl_2$	30	18	0	73.6	57.9
$CdSo_4$	90	14,18	57.5	72.5	57.5
CdS	90	18	12.5	80.0	70.0
CdS	270	16	32.5	79.0	73.6
CdO dust	30	18	5.0	75.0	57.5
CdO dust	90	7,11	35.0	60.6	48.4
CdO fume	10	18	0	0	0
CdO fume	30	18	0	19.4	5.5

[a]Each group consisted of 20 male and 20 female rats (*109,110*). The planned exposure period was 18 months, but exposure was terminated when mortality reached the 25% level. When exposure periods were different for males and females, the first of the two numbers gives months of exposure for males and the second for females.

signs of alveolar inflammation and cytotoxicity in the bronchoalveolar lavage after 1 month of exposure (*112*). The weekly exposure in this experiment was 15.2 mg Cd/m³-hr. Somewhat higher weekly exposure to CdO (24 mg Cd/m³-hr), but a more intermittent type (1.6 mg Cd/m³, 3 hr/day, 5 days/week) resulted in similar abnormalities. These peaked on the second week and declined afterwards (*114*). After 1 month of exposure, the bronchoalveolar lavage of intermittently exposed rats was nearer to control values than the lavage of continuously exposed rats.

Though hamsters and mice were resistant to the carcinogenicity of cadmium aerosols, exposure resulted in dose-dependent toxic responses in the lungs. The most common sign was bronchiolar-alveolar hyperplasia. Mean lifetime decreased in both species (*108*). The lung of Syrian golden hamsters exposed 8 hr/day, 5 days/week, to 90 μg Cd/m³ as CO for 65 weeks showed a significant increase in the volume density of proliferative areas. The damage persisted at 48 weeks after the end of exposure. CdS produced the same lesions when exposure was increased to 270 μg Cd/m³. Soluble cadmium compounds were tested with a daily 19-hr exposure to 30 μg Cd/m³ for 65 weeks. $CdCl_3$ was not effective, and $CdSO_4$ caused only slight increase in lung lesions in female hamsters (*115*).

Though there were no noticeable differences between the carcinogenic effects of different cadmium compounds in the lung (*110*), it is most likely that prostatic cancer can be induced only by soluble cadmium compounds administered by routes irrelevant to human exposure. Thus, even changing the administration from intramuscular to subcutaneous abolished the ability of $CdCl_2$ to induce prostatic cancer (*105*), probably because the delivery of Cd^2 was slower. An additional problem for the extrapolation of prostatic neoplasms induced in rats by intramuscular cadmium to exposure routes relevant to human exposure is the considerable difference between the rodent and the human prostate.

The solubility of cadmium compounds was not a decisive determinant of inhalation toxicity. At 90 μg/m³, CdS was less toxic than CdO dust, and the toxicity of the soluble $CdSO_4$ was between the toxicities of these poorly soluble compounds (*109*). Other studies found the soluble $CdCl_2$ and the poorly soluble CdO either equally toxic (*83*) or CdO more toxic to the lung than $CdCl_2$ (*112,116,117*). Both the mortality of rats (*109*) and pulmonary toxicity in hamsters (*115*) indicated that, in agreement with its higher bioavailability (*112*), CdO is more toxic than CdS.

A point of interest is the association between the carcinogenicity and the irritative-corrosive effect of cadmium. Experimental data indicate that while carcinogenicity is always the result of treatment with an irritative-corrosive dose, the administration of such a dose is not the only condition of cadmium-induced carcinogenesis. Thus, hamsters and mice respond to toxic cadmium exposure with lung damage but not cancer. Even in rats, pulmonary damage does not necessarily leads to tumor induction. One or three intratracheal instillations of 25 μg Cd as CdO caused pulmonary edema in rats, but no tumors (*133*). This seems to indicate that the continuous contact of the pulmonary tissues with cadmium (assured by continuous exposure) is more important for the induction of pulmonary cancer than the short-term presence of an acutely toxic dose.

Kinetic Considerations

The absorption of ingested cadmium ranges from 1 to 7% in different species, and it ranged from 4.7 and 7.0% in human volunteers. Cadmium in the inhaled air is in the form of an aerosol and its deposition depends on particle size. In humans, 25 to 50% of cadmium in cigarette smoke is retained (*119*). In the inhalation experiments described above, rats inhaled particles of 0.3 to 0.5 μm diameter. These are predominantly deposited in the alveolar region, the site of observed tumor formation. Assuming that in industrial exposure the median aerodynamic diameter of CdO particles is about 2 to 3 μm, deposition must be predominantly in the conducting airway and the origin of tumors bronchogenic. Limited epidemiological evidence indicates that this is the case (*85,120*).

Occupational data indicate that the pulmonary retention of CdS is lower than that of CdO of similar particle size (*119*). After 1 month of nearly continuous exposure of rats to 100 μg Cd/m³ as CdO or $CdCl_2$ or 1000 μg Cd/m³ as CdS, the pulmonary cadmium was 27, 48, and 138 μg, respectively. During a 2-month post-exposure period, the pulmonary clearance half-time (2 to 2.5 months) slowed negligible differences between exposure groups. Cadmium in the cytosolic fraction declined in proportion of the total pulmonary load. Within this fraction there was an absolute increase in cadmium bound to metallothionein (*121*). Two hours of inhalation exposure resulted in the same level of pulmonary cadmium deposition in rats exposed to $CdCl_2$ or CdO aerosol and in the first 72 hr 58% of cadmium cleared from $CdCl_2$ and 46% from CdO (*121*). The difference between the clearance of CdO and $CdCl_2$ was also negligible after intratracheal administration. After the first 12 hr clearance half-time became approximately 15 days for both compounds (*122*). The increase in clearance half-time with the extension of exposure period indicates that the fraction of pulmonary cadmium with a fast clearance becomes progressively smaller.

After absorption into blood, cadmium is mainly transported in plasma. With time red blood cells become the main carriers. The principal tissue depositories of cadmium are the liver and kidneys where most of the cadmium is bound to a sulfhydryl-rich protein (metallothionein) (*84*). Research on the mechanism of Cd-induced testicular and prostatic carcinogenesis revealed that in rats the testes and prostate are deficient in metallothionein. In the rat prostate, a cadmium-binding protein was identified that has a lower affinity for cadmium than metallothionein (*123*). Binding to metallothionein is responsible for the long clearance half-time of cadmium, 200 to 700 days in mice and rats, 2 years in squirrel monkeys, 10 to 30 years in humans (*84*).

Short-Term Tests

Studies on Salmonella, *E. coli*, or yeast have produced inconclusive results with cadmium chloride (*86,107*). $CdCl_2$ was weakly mutagenic and strongly cytotoxic in the Salmonella assay (*124*). Cadmium nitrate, which gave negative results in the plate incorporating assay, was mutagenic in the flucuation assay with *E. coli* and Salmonella TA100, though not in other strains. When, in order to prevent the precipitation of cadmium, orthophosphate was replaced by trimetaphosphate in the medium, the fluctuation assay gave negative results even with Salmonella TA100 (*47*). In the *rec* assay with *B. subtilis*, cadmium chloride, nitrate, or

sulfate were weakly positive (*125*). Cadmium chloride enhanced the mutagenic effect of *N*-methyl-*N*-nitrosourea in a synergistic manner in *S. typhimurium* either directly by modifying the nature or indirectly by inhibiting the repair of methylation damage (*124*).

In L5178Y mouse lymphoma cells, $CdCl_2$ and $CdSO_4$ consistently induced trifluorothymidine-resistant mutants in a dose-dependent manner (*51,126*). In cultured V79 Chinese hamster ovary cells, $CdCl_2$ induced mutation at the hypoxanthine–guanine phosphorybosyl transferase locus (*34*). In rat myoblasts, $CdCl_2$ induced transcriptional activation of proto-oncogens c-*jun* and c-*myc*, with a maximum in the 5 to 10 μM range (*127*).

Results of assays for the induction of chromosomal abnormalities by cadmium salts in mammalian cells have been inconsistent. CdS and $CdSO_4$ induced chromosomal abnormalities in cultured Chinese hamster cells and human leukocytes only at toxic concentrations. Pretreatment with low concentrations of cadmium protected cells against a subsequent toxic dose (*86*). There are conflicting reports on the induction of SCEs by cadmium (*34*).

In Syrian hamster cells, cadmium acetate induced transformation and decreased cloning efficiency. Cadmium acetate injected at a nearly lethal dose to pregnant Syrian hamsters on day 11 of gestation resulted in morphological changes in cells derived from progeny excised on day 13 of gestation and cloned from the third subpassage (*53*). Exposure of primary cultures of rat ventral prostate cells to $CdCl_2$ resulted in fibroblastoid and epithelial cell lines characterized by unlimited growth and changes in morphology and karyotype. Malignancies were produced by the subcutaneous inoculation of these cells into the dorsal region of rats (*128*). In the mouse embryo cell line BALB/3T3, clone A 31-1-1, $CdCl_2$ induced dose-dependent morphological changes. All the transformed foci gave rise to tumors. These appeared to be fibrosarcomas at the site of SC inoculation in nude mice (*57*).

Cadmium nitrate (0.18 to 1.8 μM) increased unscheduled DNA synthesis in cultured rat hepatocyctes (*129*) while UV-induced unscheduled DNA synthesis was inhibited by 4 μM concentration of $CdCl_2$ in cultured diploid fibroblasts derived from a normal child, in Simian kidney cells (*130*), and in HeLa S-3 cells (*131*). Zinc at 4 to 10 times higher concentrations restored at least partly the repair capability of cells (*130*). The effect of cadmium on repair may be mediated through the replacement of Zn in DNA polymerases. The high Zn:Cd concentration ratio required for restoration may reflect the higher affinity of essential binding sites for cadmium than for zinc. However, cadmium did share the inhibitory effect of zinc on the closure of UV or X-ray-induced strand breaks (*131*). In a cell-free *in vitro* system, $CdCl_2$ increased misincorporation of nucleotide bases into the daughter strand of DNA synthetized from polynucleotide templates by DNA polymerase (*64*). Cadmium also inhibited overall transcription and increased RNA chain initiation (*34*).

With one exception, soluble cadmium compounds, mainly $CdCl_2$, were used in the assays listed above. The positive assays support the view that ionic cadmium is genotoxic, but they leave open the genotoxicity of CdO and other insoluble or hardly soluble compounds.

Conclusions

The results of epidemiological studies are ambiguous (*85*). Though the interest in the carcinogenicity of cadmium started with the identification of unexpected numbers of prostatic cancer deaths in a group of exposed workers, follow-up studies failed to confirm the etiological role of cadmium. The reason for this may be either that the four cases in the United Kingdom and four cases in the United States were freak clusters or that exposure declined below the effective level. In contrast, the same follow-ups revealed increased lung cancer mortality, though with lower SMRs in the late than in the early employed groups. In a United Kingdom nickel–cadmium battery factory, excess lung cancer mortality was found ony in the group of workers employed before 1947. Thus it seems that decrease in exposure not only decreases the risk of excess lung cancer mortality but can prevent it. The role of confounding variables, such as oxyacetylene welding and nickel hydroxide in the manufacture of nickel cadmium batteries or arsenic in nonferrous smelters, cannot be ignored.

Short-term mutagenicity tests and lung cancer developed in rats exposed to cadmium aerosols have demonstrated the genotoxic and carcinogenic potential of cadmium compounds. The presence of cadmium in the cytosolic and metallothionein fractions of lung after exposure to CdO or CdS demonstrated the formation of dissolved and ionic cadmium, which were positive in several short-term tests. The *in vivo* conversion of insoluble cadmium compounds may also explain why in inhalation experiments there was no consistent difference between the carcinogenic potential of soluble and insoluble cadmium compounds.

Cadmium concentrations used in the rat inhalation experiments were several orders of magnitude higher than concentrations in ambient air but lower than industrial standards or concentrations associated with increased incidence of lung cancer in occupational cohorts. Nevertheless, the concentrations were high relative to toxic concentrations for the species. The exposure necessary for the development of pulmonary carcinogenesis was only slightly lower than the dose that killed a significant number of animals and was within the toxic range. When exposure was changed from the nearly continuous to 40 hr per week, the same atmospheric concentration became less toxic and less carcinogenic (*110*).

The overlap between the carcinogenic and nongenotoxic effects of cadmium in rats and the experimental schedule of these experiments raise some questions. One is the contribution of prolonged pulmonary inflammation to the carcinogenic process. The second question concerns the exposure schedule. Continuous exposure delivers not only 3-fold more cadmium to the lung than daily 8-hr exposure, but affects clearance (see "Kinetic Considerations") and exhausts protective mechanisms. The faster recovery of alveolar damage during intermittent, compared with continuous, exposure demonstrates the validity of this point. Based on these considerations and also on the resistance of two species, the mouse and the hamster, extrapolation from cadmium-induced lung cancer in rats to lung cancer risk in humans must be viewed with caution.

Chromium

Epidemiology

Chromium is used in a variety of different forms and occupational settings. The toxicity of chromium depends on its oxidation state and solubility. Forty years ago, Baetjer (*132*) reviewed pulmonary carcinomas associated with exposure to chromium

in chromate-producing and chromium pigment industries. As no cancer was observed in plants where exposure was restricted to bichromates, monochromate dust was considered to be the responsible agent. In the intervening years the field of studies has been extended to ferrochromium production, chromium plating, spray painting, and stainless-steel welding. There are several reviews on the epidemiology of chromium carcinogenesis (*2,133–137*). The following discussion is mostly based on comprehensive reviews published by EPA (*136*) and Yassi and Niboer (*137*).

The first epidemiological studies covered seven United States chromate production plants and indicated a nearly 30-fold increase in lung cancer mortality. Further studies covered only one or three of the seven plants and reduced the condition of minimum employment from 4 years to 1 or less. Relative risk decreased to 15, 9, and 2. In one of the studies the O/E ratio varied from 4 to 18 with increasing duration of exposure. Latency period clustered around 27 to 36 years. According to two of these studies, none of the workers was exposed exclusively to hexavalent chromium. In a British study of 2715 workers with at least 1 year of employment, lung cancer mortality showed a 2.42-fold excess between 1949 and 1977. Within this period, a change in technology (from high lime to no lime) was associated with a decrease in relative risk from 3.0 to 1.8. In German chromate works the SMRs were approximately the same, but in Hokkaido Island, Japan, in a cohort of 67 active and 487 retired chromate workers with 10 to 36 years of exposure, lung cancer mortality was 50 times higher than in the general population of Japan. A followup published 4 years later gave only one-third of this exceptionally high value. In this plant, nasal perforation was frequent, and many of the workers were heavy smokers. In a Tokyo factory where 896 workers were engaged in the production of chromium compounds (84% hexavalent and 16% trivalent), there was a 9.5-fold increase in lung cancer. Risk of lung cancer increased with length of employment. None of these studies indicated increased incidences of neoplasms at other sites (*136,137*).

The first epidemiological study of chromium pigment production workers found 3 cases of lung cancer in a group of 24 Norwegian workers with more than 3 years of employment. The three cases represented a 38-fold increase in risk compared with the lung cancer incidence of Norwegian males. A follow-up study found three additional cases. Lung cancer patients were probably exposed to 0.5 tol.5 mg/m^3 chromium during their 6 to 8 years of employment. Five of the six patients were exclusively exposed to zinc chromate (*133,135*). In studies from the United States, England, Germany, and the Netherlands, the relative risk ranged from 1.0 to 3.9. Studies from England and the United States also reported increased incidences of stomach cancer. Zinc chromate seemed to be the causative agent in both the British and one of the U.S. studies (*137*), but according to the other U.S. study, the pigment plant at Newark, New Jersey, used nine times more lead chromate than zinc chromate (*138*). A multicenter European study confirmed the tendency to elevated lung cancer risk in workers exposed to both zinc and lead chromate (*139*). An update of the Newark study, based on the vital status of 1737 workers, indicated that 30 years or more after the onset of exposure to chromate dust, but not earlier, the SMR of lung cancer increases with exposure years. Exposure years of 0, <1, 1–9, and 10+ were associated with 81, 139, 201, and 231 SMRs (*140*).

In the ferrochromium-producing industry, exposure is mostly to trivalent chromium. The relevance of reports from the U.S.S.R. on increased mortality from all malignancies in the over 50-year age group is dubious. Basic epidemiological data were deficient and exposure included hexavalent chromium and benzo[*a*]pyrene (*134,137*). A Swedish study found no increase in respiratory cancer risk among 1932 ferrochromium workers (*141*), while a Norwegian study recorded 7 lung cancers among 325 ferrochromium production workers instead of 3.1 expected. Exposures included asbestos and low levels of polycyclic aromatic hydrocarbons. No lung cancer was recorded in a group of 243 ferrosilicon workers who were similarly exposed to these agents but without exposure to chromium (*142*). In a follow-up study, the observation period was extended from 1953–1977 to 1953–1985 and the population was increased from 325 to 379 with workers employed between 1960 and 1965. In the original cohort, 3 new lung cancer cases occurred, but none in the subcohort of 54 additional men (*143*).

In the chromium-plating industry, workers are exposed to the mist of chromium trioxide (chromic acid, a hexavalent form of chromium). A British retrospective study found 109 (8.8%) deaths amoung 1238 chrome platers and 85 (6.62%) among 1284 matched-control manual workers. The corresponding numbers for total cancer deaths were 39 (3.15%) and 21 (1.63) and for lung cancer in 17 (1.37%) and 10 (0.78%). In the next 2 years, seven lung cancer deaths occurred in electroplaters and only three in controls (*144*). A second study found in a cohort of 2639 nickel/chromium platers significantly increased mortality from cancers of the lung and bronchus (72/48.1), nasal cavity (3/0.3), stomach (25/16.2), and liver (4/0.8). The O/E ratios were highest in chrome bath workers. Excesses in respiratory cancer became significant 10 years after the start of exposure and showed a positive association with the duration of chrome bath work. Although few high air concentrations were recorded (8.0, 1.6, 0.4 mg/m^3, and some workers developed nasal ulceration, the majority of measurements recorded less than 0.05 mg Cr/m^3 (*145*). In Italy, the incidences of respiratory cancer (3 vs. 0.7) and total maligancies increased significantly only in hard platers. The higher thickness of the plated chrome layer (> 5 μm versus 0.05–1.0 μm) in hard plating resulted in six times higher exposure to Cr(VI) than in bright plating (*146*). Two studies from Japan found no increase of lung cancer among chromium platers (*135,147*).

Other occupations investigated for the carcinogenicity of chromium include spray painting with chromium pigments, stainless steel welding (*137*), and cement finishing (*148*). Definite conclusions cannot be drawn because the Icelandic study on cement finishers has not been repeated in other countries, studies on spray painters are contradictory, and welders are exposed to both chromium and nickel. The latest study from a Norwegian shipyard found increased incidence of lung cancer among 783 welders. However, unlike the cohort of 279 machine shop workers or among 3610 assistants and apprentices, the increase was not significant. There was a concomitant exposure to asbestos (*149*).

There is sufficient evidence for the pulmonary carcinogenecity of chromium. The most decisive evidence is from the chromate

pigment and chromate-producing industries. Exposure is to hexavalent chromium in the chromate pigment and chromium-plating industry and to a mixture of insoluble (largely trivalent) and moderately water-soluble and insoluble (largely hexavalent) chromium compounds in the chromate-producing industry. In general, epidemiological studies have not altered the traditional view that chromates with limited solubility are carcinogens, while soluble chromates should not be regarded as carcinogens (*150,151*). Studies on carcinogenic risk in the ferrochromium industry, where exposure is predominantly to trivalent chromium, left the carcinogenic role of trivalent chromium in the unproven category (*152*). In two similarly designed Scandinavian studies, increased lung cancer risk was found in Norway where exposure was lower (*142*) and no risk in Sweden where exposure was higher (*141*). At least in one workplace of the Swedish plant, the total chromium concentration (2.5 mg/m)^3 was approximately the same or higher than that at industries with increased mortality from lung cancer. Thus, in a pigment factory, increased cancer risk became apparent in the 0.05- to 2.0-mg/m^3 exposure group (*139*) and the time-weighted average exposure of chromate production workers with increased mortality ranged from 0.11 to 0.66 mg/m^3 (*137*). Stainless-steel welders have much lower exposure to chromium. One study gives the median exposure for manual arc welding as 4 μg and for metal inert gas welding as 10 μg/m^3 (*153*). There is no definite evidence that chromium causes cancers other than in the lung (*11,154*) or for the confounding role of smoking in chromate-induced lung cancer (*155*).

Animal Experiments

Seven inhalation and four oral studies published from 1959 to 1975 and reviewed by EPA (*136*) were either negative or failed to prove the carcinogenic potential of chromium. In an additional inhalation study none of the rats exposed to Zinc chromate developed lung carcinoma (*156*). Lifetime exposure (6 hr/week, 5 days/week for 2 years) to 0.5 mg/m^3 or 25 mg/m^3 chromium dioxide (CrO_2) produced no adverse clinical signs. Pulmonary lesions were similar to those induced by nuisance dusts. None of the 106 male rats and 8 of the 108 female rats exposed to the higher chromium concentration developed pulmonary tumors. Tumors developed in female rats were devoid of true malignancy (*157*). Lifelong oral exposures (in diet or drinking water) of mice or rats to trivalent chromium oxide or chromium acetate were also without carcinogenic effect (*136*). A positive response and differentiation between the different classes of chromium compounds required administration by nonphysiological routes. Though the extrapolation of these studies to the conditions of human exposure is problematic, they allow the comparison of carcinogenic potential of zero-, tri-, and hexavalent chromium. Studies on chromium carcinogenesis have been extensively reviewed (*2,134,136,137,156*).

The development of rhabdomyosarcoma and fibrosarcoma at IM and SC injection sites was not consistent. Calcium chromate gave positive response in two of three experiments, lead chromate in two experiments, and cobalt chromium alloy in one experiment. Negative results were given by chromium powder in two experiments and sodium dichromate in one experiment (*137*). The intrapleural injection of hexavalent chromium compounds induced cancer in some but not in other experiments. With one exception, studies with intratracheal instillation gave negative results. The exception was an unpublished report reviewed by EPA (*136*). Prolonged treatment with weekly doses of 1.25 mg/kg sodium dichromate given in a single dose, but not when it was given in 5 0.25-mg/kg doses, induced 2 pulmonary adenocarcinomas and 6 squamous cell carcinomas in 80 rats. In the calcium chromate groups there were one adenocarcinoma and two squamous carcinomas. Single weekly doses of dichromate or calcium chromate induced 12 and 11 lung adenomas, respectively.

More reliable and reproducible results were obtained when the test material was implanted in a stainless-steel wire pellet into the left bronchus. Statistically significant incidence of primary lung carcinomas was seen in rats treated with calcium chromate, zinc potassium chromate, and strontium chromate. Zinc chromates consistently increased lung cancer incidence, but not always significantly. All these hexavalent compounds are sparingly soluble. Lead chromate-containing materials provided by the industry were either negative or lung cancer developed only in 1% of the implanted rats. Soluble chromates behaved like the practically insoluble lead chromate. Response to the soluble chromium oxide (CrO_3) ranged from 0 to 2 in 100 rats, and negative results were given by sodium chromate, barium chromate, and sodium dichromate. All hexavalent chromium compounds increased the incidence of squamous metaplasias, but rats given sparingly soluble chromates had more metaplasia than rats treated with highly soluble chromates. Trivalent chromium caused neither lung cancer or increased the incidence of metaplasias (*137,158,159*).

Animal studies have confirmed the intrinsic carcinogenicity of some hexavalent chromium compounds and indicate that chromium powder and trivalent chromium are not carcinogenic. Though the two latter forms have been less extensively investigated, the absence of squamous metaplasia in rats exposed to Cr(III) via implantation pellets (*159*) argues against the carcinogenic role of exposure to trivalent chromium.

Kinetic Considerations

The absorption of chromium from the gastrointestinal tract is low. In man about 2% and in rats 3 to 6% of an oral dose of hexavalent chromium is absorbed. The absorption of trivalent chromium is even poorer. Data on the urinary excretion of chromium in chrome platers indicate that dermal absorption of hexavalent chromium can be significant. The dermal absorption of trivalent chromium sulfate was below the limit of detection (*133*).

In metallurgy, most of the chromium released into the atmosphere is trivalent and in the chemical industry most is chromate dust. After the intratracheal instillation of chromate solution, about 10% of the dose is absorbed with a half-time of 1 day in rats and 2 days in guinea pigs and 80% is cleared with 30- to 40-day half-times. Clearance of chromate particles from the lung depends on solubility. Clearance is faster after exposure to sodium chromate than after exposure to lead or zinc chromate or chromic chloride. Water-soluble salts of Cr(VI) are absorbed faster than the water-soluble salts of Cr(III) (e.g., chromic chloride hexahydrate) (*160*). Ten minutes after the intratracheal instillation of 200 μg water-soluble hexavalent chromium salts (chromate or dichromate), 10% of the dose remained in the lungs, and 25% appeared in blood and in soft tissues. Ten

minutes after the intratracheal instillation of water-soluble trivalent chromium (chromic chloride), 69% of the dose was found in the lungs, and 4% in blood and soft tissues. Clearance from the trachea into the laryngopharynx and subsequent swallowing of particles was assumed to be responsible for the difference. Most of the swallowed chromium is excreted in feces (*136*).

The slower pulmonary clearance of Cr(III) may be the consequence of its more pronounced binding to extracellular macromulecules, faster conversion to insoluble forms (*133,257*), or higher toxicity to macrophages. Thus, inhalation exposure of rabbits to soluble trivalent chromium but not hexavalent chromium caused functional damage (increased reduction of nitroblue tetrazolium and decreased phagocytic activity) in pulmonary macrophages, while only Cr(VI) increased tie number of macrophages (*161*).

After IV administration, Cr(III) clears faster from blood than Cr(VI), probably because Cr(VI) is rapidly taken up by red blood cells and Cr(III) becomes bound to transferrin. Chromium cleared from blood is mostly excreted in urine and deposited in spleen, liver, and bone marrow, where concentrations are higher after the IV administration of Cr (III) than of Cr(VI). Though the primary route of excretion is urinary, a small proportion is secreted with bile and excreted in feces. In human volunteers injected with radiolabeled chromium, Cr(III) cleared from the whole body with a half-life of 22 days and Cr(VI) with 92 days (*136*). In rats, three different elimination components have been identified with half-times of 0.5, 5.9, and 83.4 days (*133*). A component with long residence time in deep body compartments is responsible for the more than 10 times higher chromium concentrations in autopsy specimens of occupationally exposed persons compared with controls. The greatest concentrations were found in the hilar lymph nodes and lungs, followed by spleen, liver, kidney, and heart with equal concentrations (*136*).

The most important difference between Cr(VI) and Cr(III) is that only Cr(VI) permeates cell membranes easily. Consequently, while Cr(VI) is highly toxic, Cr(III) has only a low toxic potential. However, Cr(VI) is easily reduced to Cr(III). Some reduction takes place in the gastric juice, and this reduction may be responsible for the low absorption of hexavalent chromium from the gastrointestinal tract. While reduction to Cr(III) outside the target cells is always a detoxification process, reduction within cells has two important consequences: the first is the intracellular entrapment of Cr(III) and the second is the prolongation of the flow of Cr(VI) from the extracellular pool into the cell (*162*). The intracellular reduction of Cr(VI) is stimulated by repeated exposure (*163*).

The speed of intracellular reduction is best demonstrated with the behavior of Cr(VI) in blood. After an IV dose of 1 mg/kg sodium chromate (VI) to rats, no hexavalent chromium was detected in blood 1 min later (*160*). Reduction takes place at any intracellular site where electron donors are present, but the primary site of reduction is the mitochondrion, where the reduction is enzyme catalyzed and NADH-NADPH dependent (*136,153*). Though the main reduction product is Cr(III), *in vitro* experiments demonstrated the presence of transient Cr(V) intermediates upon reduction by the mitochondrial electron transport chain, mainly by NADH:ubiquinone oxidoreductase and succinate:ubiquinone oxidoreductase (*162,164*). It has been suggested that mitochondrial DNA is the target of Cr(V). Reduction is consistently associated with inhibition of mitochondrial respiration. In isolated mitochondria, the inhibition of mitochondrial respiration and the reduction of Cr(VI) were increased by glutamate as electron donor and rotenone as electron chain inhibitor (*165*). There is experimental evidence for the role of Cr(III) in this inhibitory effect. Though in intact cells (hepatocytes or thymocytes), only Cr(VI) inhibites mitochondrial respiration, in digitonin-permeabilized thymocytes Cr(III) was a better inhibitor than Cr(VI) in the presence of succinate and rotenone (*162*).

The biological significance of Cr(III) is demonstrated by its roles as an essential element and in chromium hypersensitivity. In the glucose tolerance assay, the less stable Cr(III) compounds were most effective, and some Cr(VI) compounds were totally inactive. In hypersensitivity reactions Cr(III) or Cr(VI) reduced to Cr(III) acts as a hapten and forms the antigen by conjugation with protein. As in the glucose tolerance assay, the less stable Cr(III) compounds were the most active, it is most likely that the active agent is the chromic ion (*136,155*).

Short-Term Tests

Venitt and Levy (*166*) were the first to show that hexavalent chromate, but not chromium(III), was mutagenic to bacteria. The absence of either error-prone repair or excision repair did not modify the mutagenic response of *E. coli* to chromate. Dichromate increased mutation frequency in the *lacI* gene in *E. coli* (*79*). Cr(VI) and Cr(III)acetate inhibited repair in the *rec* assay with *B. subtilis* (*60*). Chromium(VI) was the most mutagenic metal ion in the Salmonella microsome assay. Only compounds insoluble in water gave negative results. Cr(III) compounds were inactive (*34*).

Cr(VI) was weakly positive with *Schizosaccharomyces pombe*, at the 8-azaguanine locus with Chinese hamster V79 cells (*136*), and in the L5178Y mouse lymphoma assay (*51*). Exposure of rat liver epithelial (ARL-15C) cells to 10 μM hexavalent chromate for 7 weeks was mutagenic at the 8-azaguanine locus, while 2 hr of exposure to 1 mM was not mutagenic (*167*). Soluble $CaCrO_4$, but not insoluble $PbCrO_4$, induced mutation at the 6-thioguanine locus in Chinese hamster ovary cells (*168*) and in diploid human fibroblastic cells (*169*). In diploid human fibroblastic cells (HFC), cellular uptake, cytotoxicity, mutagenicity, and anchorage independence were induced by the same low concentrations (0.2–1.0 μM) of soluble and insoluble Cr(VI) compounds. Insoluble Cr(III) compounds induced mutation only at the 1000-fold higher cytotoxic concentration, while a soluble form of $CrCl_3$ was not mutagenic in the same assay, though equal concentrations (0.1–10 μM) of soluble and insoluble chromium compounds in either +6 or +3 valence states induced similar frequencies of anchorage independence (*169*). Neither of the chromate compounds induced base-substitition mutation as shown by the absence of resistance to ouabain (*168*), and it is postulated that anchorage independence induced by insoluble $PbCrO_4$ or by insoluble and soluble Cr(III) compounds occurs by mechanisms not involving base substitution or frameshift mutations (*169*).

Cr(VI) induced numerous chromosomal breaks in FM3A cells from mouse mammary carcinoma. Cr(III) was ineffective (*34*). In Don Chinese hamster cells (*59*) or human lymphocytes (*170*)

strong SCE induction was given by low concentrations of Cr(VI) and weak induction by high concentrations of Cr(III). However, in P388D_1 cells, Cr(III) was a strong inducer of SCE. As human lymphocytes exclude Cr(III) and P388D_1 cells take up Cr(III) by phagocytosis, the ultimate carcinogen seems to be Cr(III) (*170*). Compared with the effective concentrations of Cr(VI) compounds, the SCE-inducing concentrations of $CrCl_3$ and Cr_2O_6 in Chinese hamster V79 cells were 300- and 1000-fold higher in terms of chromium (*171*). Crystalline Cr_2O_6 also caused concentration-dependent accumulation of cells in the G_2 phase and increased up to 10-fold mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in V79 Chinese hamster cells. Phagocytized crystalline chromic oxide was regularly identified in electron micrographs (*172*).

Cr(III) was negative in a mammalian cell transformation assay, but Cr(VI) gave consistently positive response (*8*). In agreement with epidemiology or *in vivo* carcinogenicity data, insoluble $PbCrO_4$, but not the soluble $CaCrO_4$ induced morphological transformation and anchorage independence in C3H10T1/2 Cl mouse embryo cells (*168*). Solubility was not a factor in the ability of chromate to transform Syrian hamster embryo cells (*55*), BALB/3T3 clone A31-1-1 cells (*57*), or diploid human fibroblastic cells (*169*). In the last system, Cr(VI) and Cr(III) were equally effective (*169*). Neoplastic transformation was also induced in Syrian hamster embryo cells grown in culture on a 10 to 50-nm thick layer of evaporated pure chromium (*173*). For the transformation of rat liver epithelial cell line (ARL-15C_1), the optimum concentration and exposure time were 1 mM K_2CrO_4 and 2 hr. At this exposure level Cr(VI) was not mutagenic (*167*).

Sodium dichromate given to 14-day-old chick embryos had no effect on the expression of mRNAs for constitutive genes (e.g., for albumin), but increased the basal expression of mRNAs for inducible genes (e.g, for cytochrome PB_1 and P-450) and suppressed the expression of the same genes when induced by a phenobarbitone analog. The time courses of these two opposite effects were bimodal: the first peak corresponded to rises in Cr(VI)-induced DNA damage, repair, and chromium bound to DNA, while the second peak correlated closely with the formation of DNA cross-links and strand breaks. The authors suggest that "chromium, like cisplatin, may initially produce a DNA monoadduct that subsequently leads to cross-link formation and both types of chromium(VI)-induced lesions have a significant effect on the expression of targeted genes" and that "the expression of inducible genes is more sensitive to the effect of carcinogen treatment than is constitutive gene expression *in vivo*" (*174*).

DNA-protein cross-links occurred in proportion to the concentration of chromate in several mammalian cells exposed to Cr(VI) salts. However, Cr(III) but not Cr(VI) induced DNA-protein cross-links in isolated or solubilized nuclei. This finding indicates that Cr(III) is the active form (*175*). In agreement with this view, it has been shown that Cr(III) forms Cr-nucleotide complexes with dissolved nucleic acids. A similar complex was formed in the presence of Cr(VI) only when Cr(VI) was reduced to Cr(III) by excess glutathione (*176*). However, when plasmid pBBR322 DNA was incubated with DNA polymerase, added Cr(III) did not induce strand breaks but Cr(VI) reduced by peroxide to Cr(III) did. Strand breaks did not occur when Cr(VI) was reduced by GSH or when the incubation medium contained peroxide and superoxide dismutase. The authors concluded that this specific effect is linked to the formation of superoxide and hydroxyl radicals from the decay of peroxochromium(V)complexes (*177*). The generation of the hydroxyl radical and singlet oxygen and cleavage of DNA was observed when DNA was incubated with sodium chromate in the presence of hydrogen peroxide (*178*). Chromium(VI) was co-mutagenic with UV in CH V79 cells (*179*) but did not inhibit the repair of UV-induced damage in HeLa S-3 cells (*131*).

It has been shown that chromium in several oxidation states (II, III, or VI) decreased the fidelity of *E. coli* DNA synthesis (*64,180*). At micromolar concentrations (0.5–5.0 μm), Cr(III) activates polymerase, probably through the binding of Cr^{3+} to polymerase-template. This effect is specific for chromium and manganese (*181*).

Conclusions

Chromium is the only metal that is convincingly genotoxic. Though the current evidence is insufficient to pinpoint carcinogenic and noncarcinogenic chromium compounds, it is apparent that either only sparingly soluble chromates are carcinogenic (*150*) or sparingly soluble chromates are more carcinogenic than soluble chromates, and the carcinogenic potency of chromates is some order of magnitude over the potency of trivalent chromium compounds (*137*).

The difference between chromates and chromic compounds seems to reflect the penetrability of membranes for hexavalent but not for trivalent chromium. The variation in cellular uptake also explains the strong genotoxic effect of Cr(VI) in *in vitro*-exposed, intact cells and the negative or week response to Cr(III).

The difference between the carcinogenic potency of sparingly soluble and soluble chromates indicates that the prolonged exposure of target cells to chromium slowly dissolved from a pool of chromium particles is more favorable for the carcinogenic process than high but temporary exposure to instilled or injected soluble compounds. Though exposure to sparingly soluble chromates has the carcinogenic potential, both the kinetics of chromates and the reaction of Cr(III) with DNA indicate that the ultimate carcinogenic species is most likely Cr^{3+}

Lead

Epidemiology

Lead in its compounds has either +2 or +4 valencies. The bivalent form is the more stable, and it is the main form of inorganic lead associated with occupational and environmental exposure. Contrary to the hematologic, neurologic, and renal effects of lead, the interest in lead carcinogenicity is relatively new. Epidemiological studies, the subject of several reviews (*2,182,183*), mainly focused on the association between lead exposure and cancer among workers engaged in the production of lead (smelters) and storage batteries. (Lead chromate production is discussed in the section on chromium.)

Dingwall-Fordyce and Lane (*184*), in the introduction of their study on lead workers, wrote that according to the report of the Registrar-General in the period of 1949 to 1953, there had been 7 deaths attributed to malignant neoplasms among 1200 lead burners (burning PbS containing ore to produce PbO). This

figure indicated a rather better mortality experience than that which occurred among the general population of England and Wales. In their own study on men becoming eligible for retirement between 1926 and 1960, they found that 25 years of exposure to lead (100 to 250 μg Pb/L urine) in a lead acid battery plant was not associated with excess of deaths from malignant neoplasms. There was a slight but not significant excess in ancillary workers with lead urine values within normal range. A follow-up (*185*) of 1898 pensioners from four lead acid battery factories found no increase in mortality from malignant neoplasms between 1925 and 1976. The analysis of 553 deaths that occurred before retirement showed that 63 men died of neoplastic diseases, while the expected was only 48.58. The excess of malignant neoplastic diseases of the digestive tract was even higher: 21 compared with 12.56 expected. Nevertheless, the authors concluded that these excesses were not the consequence of prolonged exposure to lead. First, there was no excess mortality from cancer in pensioners, and the excess mortality of men in service was almost completely confined to the period of 1963 to 1966. Such distribution is unlikely to be related to continuous exposure to lead (*185*).

The study population of these lead-acid battery factories belonged to the pension scheme of an international conglomerate with a number of manufacturing facilities. The records of this scheme were the main source of data for a case–control study of men who died between 1926 and 1985. The lead group consisted of 867 men who had relatively high occupational lead exposure, and the control group consisted of 1206 men with low or no lead exposure. In the lead group there was no statistically significant excess in the number of deaths from malignant neoplasms, either in general or at specific sites (*186*).

The mortality data of 3832 workers first employed before 1967 in a Swedish copper smelter were followed from 1950 to 1981. From the total cohort, 457 workers were selected who had been employed at sites with considerable lead exposure for at least 3 years. Based on regular blood lead measurements, this subcohort was divided into high-mean and low-mean or high-peak and low-peak exposure groups. Mean blood lead concentration decreased from 582 μg/L in 1950 to 336 μg/L in 1974. The study indicated that even under conditions regarded as unacceptable today, exposure to lead did not increase the incidence of neoplasms significantly. No association has been shown between exposure to lead and renal neoplasms (*187*).

One of the largest studies, with several follow-ups, was conducted in the United States on a group of 7000 workers in lead-battery and lead-producing industries. The first study was published in 1975 and reported a small excess of mortality from cancers of the digestive and respiratory systems for the period of 1947 to 1970 (*2*). The latest follow-up study by Cooper et al. (*188*) extended the observation period to the end of 1980 and included 4519 battery plant and 2300 lead production workers. SMRs were calculated from the death rates of U.S. white males. The SMRs in the two cohorts were 113 and 113 for all malignant neoplasms, 124 and 125 for lung cancer (including bronchus and trachea), and 168 and 146 for stomach cancer. The excesses were significant only in the cohort of battery workers. The SMRs were also high for liver cancer but there were only 5 deaths in the cohort of battery workers and 4 in the lead production workers (compared with 34 and 9 for stomach and 109 and 41 for lung cancer). Particularly in the lead production plants (one primary and two secondary smelters and three recycling plants), there were confounding exposures to arsenic, cadmium, and SO_2. SMRs for the gastric cancer mortality may have overestimated risk because U.S. rates for white males were lower than in the state of Pennsylvania or more importantly in Philadelphia (where the largest battery plant was located) and in the country of origin of most of the foreign workers. In battery plants, 13 of 34 and in production plants 6 of 9 workers who died from gastric cancer were immigrants. Other uncontrolled confounding variables were alcoholic intake for stomach cancer and smoking for respiratory cancer. The elevation of lung cancer deaths fell within the range attributable to a slightly heavier-than-average smoking pattern. Monitoring data was far from satisfactory. First, they did not cover periods when exposure was probably the heaviest; second, they were available for less than one-third of the population. Thus it is not "justified in extrapolating from the observed patterns of mortality to risk estimates that are relevant to lead exposure levels currently used or recommended as standards" (*188*).

In another study, 6 deaths from renal cancer were identified when 2.94 were expected in a cohort of 1987 smelter workers in Idaho. The excess was not significant (*189*). In an Australian study, the age-standardized mortality analysis showed a deficit of cancer deaths between the group of 242 smelter workers with a history of lead poisoning during 1928 to 1959 and a group of 695 other male decedents from the same smelter. The substantial excess in the number of deaths from both chronic renal disease and cerebral hemorrhage (*190*) suggests that betweeen 1938 and 1959 the diagnosis of lead poisoning (and suspension from work associated with lead exposure) was based on more severe defects in hemesynthesis and higher lead blood levels than at present. There were no significant increases in mortality from malignancies in 2510 males hired from 1952 through 1977 by a chemical plant where potential exposure included inorganic lead and tetraethyllead (*191*).

No evidence has been provided on association either between parenteral lead exposure and the risk of Wilm's tumor (adenocarcinoma with mixed carcinomatous elements that occur fetally and may lie dormant for years) or lead concentration in drinking water and cancer risk (*192*).

Emission of lead into the atmosphere of production and battery plants is either lead dust (mainly oxide) or vapor from the molten metal. As vapor in contact with air is rapidly oxidized, at least in these two industries, workers are mainly exposed to lead oxide. Cooper et al. (*188*) reported urinary and blood lead concentrations for less than one-third of the workers. Mean urinary lead concentrations were 130 μg/L in battery and 173 μg/L in production plant workers. The corresponding numbers for blood lead were 627 μg/L and 797 μg/L.

In a review on the carcinogenicity of lead, Kazantziz (*192*) did not amplify on a previous IARC evaluation that acknowldged the absence of adequate human data on the carcinogenicity of lead. Several studies are negative, and small excess risk in other studies could be explained by confounding factors. Nevertheless, increased risk observed is some studies keeps concern about the human carcinogenicity of lead on the agenda.

Animal Experiments

In the first experimental demonstration of lead-induced carcinogenesis, rats were injected once a week subcutaneously with

1 mL of 2% lead phosphate suspension (15.3 mg Pb) for 2 to 36 weeks. Nineteen of 29 rats which were alive 10 months after the first treatment developed tumors in the renal cortex. Most of the tumor-bearing animals had multiple, expansive, but encapsulated tumors, diagnosed as adenomas, but three had tumors which infiltrated the neighbouring tissues and two of the three had metastases (*193*). In addition to this study, EPA (*182*) reviewed several others which showed that intramuscularly injected lead caused injection-site fibrosarcomas and rhabdomyosarcomas and that lead, at toxic oral doses, is tumorigenic. The tumors were mostly adenomas and occasionally malignant neoplasms. Most of these studies used lead acetate or subacetate administered in the diet to rats at concentrations of 1000 or 10000 ppm. Mice seemed to share the sensitivity of rats, while hamsters were resistant to the tumorigenic effect of lead acetate. According to EPA reviews (*182*), these studies did not provide much useful information, as they do not allow the determination of the relative carcinogenic potency of lead and they had some deficiencies in protocol and design.

EPA (*182*) considered that the question of lead carcinogenicity had been addressed in a "definite manner" by a 2-year feeding study that used two species, two sexes, and a wide range of lead acetate concentrations (*194*). Unfortunately, both the description of methodology and the presentation of data lacked important details. Lead concentrations ranged from 0 to 2000 ppm for rats of undefined strain and 0 to 500 ppm for beagle dogs. A table gives the number of rats in each sex for each dietary level, percent mortality, and percent renal tumor incidence. Whether tumor incidence refers to total or surviving rats is not defined, and information given on the nature of tumors is a single sentence which states: "Most of the tumors were adenomas derived from the tubular epithelium" (*194*). None of the dogs developed renal tumors. No renal tumor was observed in rats at 100 ppm or below or in female rats at 1000 ppm or below. Renal tumor incidences in male rats were 10% at 548 ppm, 50% at 1150 ppm, and 80% at 2100 ppm. In female rats the incidence rates were 0% at 1150 ppm and 35% at 2100 ppm. Other pathological changes (undefined) were seen in rats at 500 ppm or higher levels. The hematological effect of lead was evident at a dietary lead concentration of 50 ppm. Increase in mortality above the high background levels of 37 and 50% in male and 34 and 35% in female rats was not consistent, but it reached 80% in males in the highest exposure group (*194*).

Lead acetate and male rats were used in three other experiments. Mortality from acute toxicity was 19/50 in Fischer rats exposed to 10,000 ppm dietary lead. Surviving rats sacrificed at 16, 24, or 36 weeks showed signs of renal hyperplasia, and one of five rats sacrificed at 52 weeks had renal adenocarcinoma (*195*). In Wistar rats, 24 weeks of exposure to 6300 ppm lead produced renal tumors composed of basophilic, chromophobic, and oncocytic cells. Exposure to 3150 ppm had no tumorigenic effect (*196*). In another experiment, male Sprague-Dawley rats exposed to 2600 ppm lead in drinking water were killed when moribund or at 76 weeks. Renal tumors, identified as tubular carcinomas, developed in 13 of 16 rats (*197*).

Groups of 30 male Wistar rats were fed on a diet containing 1% lead subacetate (8000 ppm Pb). Only one controls and seven exposed rats died before the end of the 18th month. None of the controls had renal tumors, while 8.7% of the exposed rats had adenocarcinomas and 47.8% had adenomas. The earliest tumor appeared at 58 weeks. The supplementation of the lead diet with 0.3% calcium acetate increased the incidence of adenocarcinomas to 19.2% and the incidence of adenomas to 61.5%, and it decreased body weight and nearly halved lead concentrations in kidneys (*198*).

In strain A mice, 24 (twice weekly) IP administrations of 6.25 mg/kg lead subacetate (basic lead acetate) doubled the high background incidence (37%) of pulmonaryadenomas (*199*). The induction of lung tumors was prevented by the IP administrstion of magnesium or calcium acetate (*200*).

Synergistic effects were reported in the kidneys of rats given lead acetate and *N*-nitroso-*N*-(hydroxyethyl)ethylamine or *N*-(4'-fluoro-4-biphenyl)acetamide orally and in the lungs of hamsters given lead oxide and benzo[*a*]pyrene given intratracheally (*50*). In *N*-(4'-fluoro-4-biphenyl)acetamide-treated rats, 10000 ppm dietary lead accelerated the onset of renal lesions including the development of renal carcinoma (*195*). No syncarcinogenicity was observed when rats were given 2600 ppm lead in their drinking water for 72 weeks and, from 23 weeks onward, ethylurea (EU) and sodium nitrate ($NaNO_2$) in food. Lead did not increase the incidence of lymphosarcoma, and EU/$NaNO_2$ decreased the incidence of renal tumors (*197*). Synergistic or inhibitory effects were not observed after prolonged exposure to lead acetate (6400 Pb in diet) and *N*-nitrosodiethylamine, though both act on the same segments of renal tubules (*196*). In Swiss mice, 15 weeks of exposure to 50, 200, or 1000 ppm lead (as lead acetate) in drinking water did not effect urethan-induced lung adenoma formation. When lead was administered at two levels (50 and 1000 ppm) for 280 days without any additional treatment, mortality from spontaneous lymphocytic leukemia of thymic origin, prevalent in this strain of mice, increased by low exposure from 25/50 to 34/50. The difference was significant. However, the increase of mortality from 35/50 to 38/50 by a 40-fold increase in exposure was not significant, though lead concentrations increased 9-fold in kidney and 5-fold in liver (*201*).

Animal studies leave no doubt that inorganic lead is tumorigenic. Some species (e.g., hamsters and dogs) did not respond with tumors, but in rats, prolonged oral exposure to lead consistently induced renal tumors. In the absence of appropriate experiments, there is no evidence that inhalation exposure to inorganic lead is carcinogenic, and there is no good evidence that lead can induce tumors at other sites. The finding that IP lead subacetate increased the incidence of lung adenomas (*199*) in mice may be valid only for a particular strain which has a high background incidence of this tumor. The development of malignant tumors mostly occurred in rats when exposure was extremely high, e.g., 10000 ppm soluble lead in food or drinking water. Thus, inorganic lead belongs to the group of compounds that must be given at or near to the maximum tolerated dose and well above the dose acutely toxic to the target. There is some indication that the site of acute renal damage is the site of neoplastic transformation (*196*).

Kinetic Considerations

The gastrointestinal absorption of ingested soluble lead is 4 to 21%. In fasting human subjects, due to the absence of calcium and phosphate in the gastrointestinal tract, the absorption of lead can be as high as 60%. Children age 3 months to 8 years absorb

substantially more than adults. The same difference is seen in animals: adults absorb 5 to 10% and young animals 50%. Absorption of lead acetate through the intact skin is from 0 to 3% and more from scratched skin. The dermal absorption of organolead compounds can be substantial (*182,202*).

Data tabulated by EPA (*182*) indicate that about half of inhaled lead is retained in the respiratory tract and most of the inhaled lead (e.g., lead oxide) is cleared from the lung within 24 hr. Such a rapid clearance explains the lack of lead accumulation in the lung of deceased lead workers. The exception is the accumulation of lead in workers exposed to lead sulfide (*202*).

Absorbed lead in blood is located mainly in erythrocytes, and it is distributed to soft tissues where liver and kidneys accumulate the highest concentrations. The initial distribution is followed by redistribution between the exchangeable compartment (blood and soft tissues) and the storage compartment, essentially the skeleton, where the concentration can be 10 to 40 times higher than that in liver or kidneys. In animal experiments, low dietary calcium increased the proportion of absorbed lead in soft tissues. High dietary calcium decreased absorption, and more lead was carried with calcium into bones (*203*).

The clearance half-time of lead from blood and soft tisues is about 3 to 4 weeks and from the skeleton is 20 years (*204*). Excretion is mainly in urine. Nearly all the deposited lead must pass through the kidneys where interstitial fibrosis develops when blood lead concentrations are over 600 μg/L (*203*). In rats exposed to 500 ppm dietary lead, the blood concentration of lead exceeded this value (*194*). Renotoxicity may be an important factor in the tumorigenic effect of lead.

Lead has been shown to interact with essential elements, particularly calcium, zinc, and iron (*203*). The effect of calcium on the absorption and distribution of lead is well documented (see above). The carcinogenic interaction of calcium acetate with lead acetate is not clear. Differences in route of administration, species, and tumor sites explain why calcium acetate prevented the carcinogenic effect of lead in one experiment and potentiated it in another experiment. The potentiation of lead carcinogenicity by dietary calcium acetate was noticeable in spite of a decrease in renal lead accumulation (*198*). The replacement of calcium by lead ions at functionally significant binding sites may be more important than the effect of calcium on the absorption and distribution of lead. Lead may substitute for Ca^{2+} in the calcium-specific protein calmodulin, which stimulates a variety of enzymes including calcium-dependent protein kinase (*10*). Protein kinase C is a receptor for tumor promoters, like phorbol esters, and the gene products of some proto-oncogenes, such as c-*fos* and c-*myc*, are potential targets of protein kinase C action (*205*). It is noteworthy that lead in picomolar concentrations stimulates protein kinase C (*206*).

Short-Term Tests

Reviews on the genotoxicity of lead indicate that inorganic lead is not mutagenic in microbial or mammalian systems (*1,2,192*). There are conflicting reports on chromosome aberrations in mammalian cells exposed *in vivo* or *in vitro* to inorganic lead or in lymphocytes from humans exposed to lead (*1,182,183*). One study reported that 0.5 mM concentration of lead sulfide and lead nitrate increased mutation at the HRPT locus in Chinese hamster V79 cells by 6-fold. This concentration was cytotoxic (50% inhibition of cloning ability) for the readily phagocytized insoluble PbS but not for the more soluble $Pb(NO_3)_2$ (*207*). Increase in SCE was reported in workers exposed to lead, but not in children with elevated blood lead level and other chromosomal aberrations (*182,192*).

Lead acetate induced morphological transformations in Syrian hamster embryo cells in a dose-dependent manner. Transformed cells cloned and administered to nude mice formed fibrosarcomas (*1,207*). Lead oxide enhanced the simian adenovirus (SA-7)-induced transformation of Syrian hamster embryo cells (*54*).

Hepatic DNA synthesis was increased in rats after the IV injection of 10 μmole/kg lead nitrate. Maximun was reached at 36 hr and synthesis returned to normal at 72 hr (*208*). In Syrian hamster embryo cells 200 μmole lead acetate caused a small increase in DNA repair (*209*) and in a cell-free system with natural DNA templates lead chloride inhibited the fidelity of DNA synthesis (*64,180*).

Conclusions

Knowledge of the carcinogenicity of lead is characterized by negative epidemiological studies, the dominance of adenomas over malignant neoplasms in animal studies, and the paucity of positive short-term tests. Lead compounds are not mutagenic, and so far, lead-induced morphological transformation has been observed only in Syrian hamster embryo cells exposed to lead acetate. The crucial evidence for the tumorigenicity of lead is given by bioassays. The first experiment that used a sufficient number of controls and a wide range of dietary concentrations was a 2-year feeding study. It resulted in mostly adenomas in male rats exposed to 500 to 2000 ppm lead and female rats exposed to 2000 ppm lead (*194*). In a similar feeding study, much higher exposure (8000 ppm) induced renal carcinomas only in 8.7% of male rats (*198*).

The disagreement between the epidemiological evidence and animal experiments may be due to differences in species sensitivity or exposure. Difference in sensitivity exists between rats and hamsters or dogs, and it may exist between rats and humans. However, even assuming that the rat mimics the sensitivity of man, the negative outcome of epidemiological studies is not surprising. As the daily food consumption of rats approximates 5% of the body weight, the experiment of Azar et al. (*186*) indicates that in male rats the daily ingestion of 25 mg Pb/kg (500 × 0.05) and in female rats the daily ingestion of 100 mg Pb/kg is tumorigenic. The former value is about 3500 to 5000 times the average daily human dietary intake (*210*), five times the smallest dose that can produce acute human poisoning (*211*), and about 100 times the daily oral dose, which after 2 or 3 months forced the hospitalization of a women with severe anemia (27% hematocrit), arthralgia, and peripheral and central nervous disorders (*198*). Considering 10% gastrointestinal absorption and 30% absorption from the inhaled lead particles, a worker of 70 kg body weight would have to be exposed to 70 mg/m³ lead during the 40 hg working week to receive the equivalent absorbed daily dose. This concentration is 1400-fold higher than the present permissible exposure limit in the United States and would send the hypothetical worker to the hospital within weeks if not days.

Nickel

Epidemiology

Nickel forms a series of compounds with oxidation states from −1 to +4, but the most prevalent and commercially and environmentally significant form is +2. It has many industrial applications, but its primary production presented the most serious health problems. The first report that implicated nickel as a carcinogen was the annual report of the Chief Inspector of Factories and Workshops in England and Wales for 1932 [see Doll et al. (*213*)]. The unusually large number of cases of nasal and lung cancer came from a nickel refinery at Clydach, Wells, which refined nickel matte (impure metal sulfide) made in Sudbury, Ontario. After milling the matte and converting copper and nickel sulfides to oxides by calcination, the two oxides were separated by the Mond process. Nickel was carbonylated to volatile (boiling point 43.2°C) nickel carbonyl followed by decomposition at 60°C. At first the highly toxic nickel carbonyl was blamed for the carcinogenic effect, and the cancer was recognized as occupational in origin only in men working in a factory where nickel is produced by decomposition as a gaseous compound. However, based on epidemiological studies this definition and the role of nickel carbonyl in nickel carcinogenesis was discarded in favor of the role of nickel-containing dust (*213*). The epidemiology of nickel carcinogenesis has been recently widely reviewed (*2,214–217*) and the International Committee on Nickel Carcinogenesis in Man (*218*) updated and analyzed data from 10 previously studied cohorts and supplemented them with exposure estimates for individual workplaces. These estimates were derived from the knowledge of chemical processes, historical records of plant operations, and available environmental measurements. The following section is mainly based on the EPA document (*214*) and the report of the International Committee (*218*).

Successive changes in technology reduced exposure to nickel at the Clydach plant. A study was a dramatic drop in cancer risk. A study that followed mortality to the beginning of 1972 found that in men first employed before 1930 the O/E ratios were for the carcinoma of nasal sinuses 56/0.195 and for the cancer of lung 137/21.98. No deaths were attributable to nasal sinus cancer in men who started work after 1924 and the O/E for lung cancer decreased to 1.5 (8/5.46) in men who were first employed in 1930 or later (*213*). The latency period was 20 to 50 years for nasal cancer and somewhat shorter for lung cancer. Any time spent in the furnace area or more than 5 years spent in the copper sulfate area increased risk (*214,218*). Data analysis indicated that at Clydach, increased lung and nasal cancer risks were associated with exposure to sulfidic and possible oxidic nickel and that exposure to soluble nickel accentuated risk, though there was little evidence that soluble nickel alone was carcinogenic. Until the replacement of the Mond process, the concentration of these three nickel species exceeded 10 mg Ni/m³. There was no evidence that metallic nickel increased the risk of lung or nasal cancers (*218*).

Similar high exposure to oxidic and sulfidic nickel was present in INCO (International Nickel Company) sinter plants at Copper Cliff and Port Colborne, Ontario, and in the Falconbridge Refinery in Kristiansand, Norway. Some men in these plants were also exposed to soluble nickel. Exposure at other places was low (<1 mg Ni/m³), with the exception of medium sulfidic nickel exposure (1–4 mg Ni/m³) of one of the 20 cohorts at Falconbridge, Ontario, and one of the 17 cohorts at Huntington Alloys, West Virginia (*218*).

Sintering (heating a powder to produce a solid mass) was part of matte processing at Copper Cliff and sintering was used with calcining at Port Colborn. The O/E ratios were 41/9.68 and 50/17.9 for lung cancer and 2/0.13 and 16/0.2 for nasal cancer. Increases in larynx and kidney cancer deaths, though not significant, were found only in the plant where sintering was coupled with calcination (Port Colborne). In a third plant, located with the Copper Cliff plant in the Sudbury area, sintering was part of the smelting process. At this Coniston plant no cases of nasal, larynx, or kidney cancers were identified and the O/E for lung cancer was 5/1.75. Risks among INCO nonsinter workers were not elevated significantly (*214*). The latest follow-up covered 54,509 INCO workers who had been employed between the beginning of 1950 and the end of 1976 for any period of time in sinter plants or 6 months or more in other workplaces. Analysis of mortality data indicated that the risk of respiratory cancer increased with the duration of service in sintering plants (Copper Cliff or Coniston in the Sudbury area) or in the Calcining and Sintering Department of Port Colborne and incidences of lung and nasal cancers increased when the period after first employment was longer than 15 years (*219,220*). The International Committe (*218*) also tabulated O/E ratios for lung and nasal cancers. At Copper Cliff, Coniston, and Port Colborn, the O/E ratios of sinter workers were 63/20.51, 8/2.67, and 72/30.4 for lung cancer and 6/0.17, 0/0.02, and 10/0.24 for nasal cancer, respectively. In the Sudbury area the O/T of nonsinter workers for lung cancer was 493/444.68 and for nasal cancer 4/3.4, while in the Port Colborn area the corresponding numbers were 30/32.32 and 0/0.25 (*218*).

In the Falconbridge Refinery, Norway, cancer cases were identified in a group of 2247 workers who were employed for at least 3 years and were alive in 1953. Betwen 1953 and 1979, there were 82 lung cancer, 21 nasal cancer, and 5 laryngeal cancer cases. Four of five laryngeal cancer cases came from the roasting-smelting group (O/E = 4/0.6). Exposure in this group was mainly to particulate nickel subsulfide and oxide and O/E ratios for lung cancer (19/5.2) and nasal cancer (5/0.1) were high. The highest lung cancer O/E (40/7.3) occurred in electrolytic workers exposed to aerosols of soluble nickel sulfate and chloride. However, as leaching of the calcined matte was carried out in the electrolytic department, exposure may have included nickel subsulfide and oxide (*214,216*).

In the group of 266 men who had worked at least for 1 year near calcinators at Huntington, there was a significant excess of nasal cancer deaths (2 versus 0.08 expected) without excess of lung cancer. No nasal cancer occurred in the cohort hired after 1947 (*218*).

At Falconbridge Ltd. (Ontario), no nasal cancer was observed in men in any of the job categories. Risk of lung cancer increased significantly in two exposure categories, mines and service, and nonsignificantly in mills and smelters (*214*). The extension of the follow-up period from 1950–1976 to 1950–1984 found significant excess risk only for lung cancer death, with 114 observed versus 84.6 expected. With the exception of mines and smelter, there was little evidence of any association between lung

cancer risk and duration of exposure in any other exposure categories (*218*). No cases of nasal or lung cancer were identified in workers of a hydrometallurgical nickel refinery at Fort Saskatchewan, Alberta. Exposure was to partially oxidized sulfur compounds of nickel (including Ni_3S_2 and amorphnus NiS) as well as nickel sulfate and nickel powder (*217*).

Nickel in some geological formations is associated with silicate (oxide ore) instead of sulfide. Studies carried out at Hanna Nickel Smelting Company, Oregon, indicated no excess risk from lung, nasal, or laryngeal cancer in miners or smelter workers. However, all atmospheric cnncentration values were below the 1.0 mg/m³ TLV value (*214*). In an oxide ore refinery at Fort Saskatchewan, Alberta, two lung cancer cases were detected, but they occurred in the maintenance group. However, a significant excess of lung cancer deaths was reported from a similar refinery in the South Pacific Island of New Caledonia (*216*).

Exposure to nickel in nickel-using industries is usually below 0.1 mg/m³. The average air concentration in the personal air samples of 123 stainless-steel welders was 0.093 mg Ni/mg³ (*221*). Epidemiological studies indicate low or no excess of cancer risk in welders, stainless-steel production workers, metal polishing and plating workers, and nickel alloy manufacturing workers. Slight but significant increases in lung cancer deaths were observed in high-nickel alloy plant workers, die-casting and electroplating workers, but owing to the presence of confounding variables the causative role of nickel was dubious (*214,216,217*).

The available epidemiological evidence clearly indicates that exposure to nickel-containing dust present in the working environment can cause cancer in nickel production workers 5 to 25 years after the onset of exposure. There is convincing evidence for excesses of cancer in lung and nose, but not at other sites (*218*). The interaction of smoking with nickel was an important factor in lung cancer deaths in the Norwegian refinery (*222*), but smoking histories were usually not collected. Cancer is not the only adverse effect of nickel. The targets of carcinogenicity are also the target of noncarcinogenic effects and include chronic pulmonary irritation, hypertrophic rhinitis, and nasal sinusitis, all of which frequently occur in exposed populations (*223*).

The International Committee on Nickel Carcinogenesis in Man (*218*) concluded that respiratory cancer risks are primarily related to exposure to soluble nickel at concentrations in excess of 1 mg Ni/m³ and to exposure to less soluble oxidic and sulfidic forms at concentrations greater than 10 mg Ni/m³. Much of the evidence of increased lung and nasal cancer risk was seen in refinery workers exposed to large amounts of nickel species through processes used in the past. For example, the reduction oi airborne levels of oxidic and sulfidic nickel dust and soluble nickel mist resulted in the disappearance of respiratory cancer risk at Clydach where in the calciner the average oxidic nickel concentration decreased from 20 to 6–8 mg Ni/m³ and the sulfidic nickel from 6–9 to less than 3 mg Ni/m³.

Animal Experiments

Of all the metals, nickel has the largest literature on experimental carcinogenesis. The subject has been covered by several reviews (*2,4,214,217,224,225*).

Sarcomas have been produced at injection sites (mostly IM and SC but also intrafemoral, intrapleural) by several nickel species. There were great differences in the carcinogenic potential of different nickel compounds. Thus, Ni_4FeS_4 (sinter matte), nickel subsulfide, crystalline NiS, NO, and NiS_2 induced local sarcomas in all or nearly all treated rats. Ni powder and $Ni(OH)_2$ had medium and insoluble amorphous nickel sulfide low carcinogenic potency. Black nickel oxide (Ni_2O_3), colloidal $Ni(OH)_2$, and the soluble nickel sulfate and nickel chloride were ineffective (*4,214,217*).

There are species and strain differences in response to the IM injection of nickel subsulfide. The order of rhabdomyosarcoma formation in different species was rats > mice > hamsters and in different strains of rats, Hooded > Wistar > Fischer > Sprague-Dawley. A single intrarenal injection of nickel subsulfide induced renal tumors in Fischer, Wistar-Lewis and NIH Black, but not in Long-Evans rats. In rats, intratesticular,intraocular, and intrapleural, but not intrahepatic or submaxillar, injection of nickel subsulfide induced local tumors (*214*). Although tumors induced by IM injection were rhabdomyosarcomas, they were mainly malignant fibrous histiocytomas after SC injection (*226*).

The epigenetic origin of Ni_3S_2-induced injection-site tumors is suggested by the long period of cellular irritation, muscle cell degeneration, and regenerative changes that occur before tumors develop (*227*). Three weeks after intrarenal injection of crystalline nickel subsulfide particles, subcapsular granulation, linear scars, diffuse black deposits along the needle track, and focal tubular regeneration were seen. Twenty-one weeks after treatment, all six rats had renal malignancies, mainly sarcomas. Oncogene amplification was documented in two tumors, and prominent marker chromosomes were seen in the karyotypes of three of the six neoplasms. Blood hematocrit values were increased during 2 to 36 weeks postinjection (*228*).

No carcinogenic effect was observed by lifetime exposure to 5 ppm nickel acetate in drinking water in mice and rats or by 2 years of exposure to 100, 1000, and 2500 ppm dietary nickel (as nickel sulfate) in rats and dogs. The lack of carcinogenicity at the higher concentrations is noteworthy because nickel decreased growth rate in rats, and the highest concentration decreased growth and lowered hematocrit and hemoglobin values (*214*). The carcinogenicity of skin exposure to nickel has not been studied. The thrice-weekly IP injection of 15 mg/kg nickel acetate for 8 weeks increased the incidence of lung adenomas in strain A mice (*200*), while the once-weekly IP injection or intratracheal instillation of 0.16 mg/kg nickel subsulfide for 5 weeks was ineffecive (*229*).

There are few data on intratracheal instillation. No lung tumors were observed in hamsters given NiO particles or in rats treated with nickel powder (*214*). The weekly administration of 1.1 mg/kg nickel subsulfide for 4 weeks by intratracheal instillation did not increase the incidence of malignant or nonmalignant tumors in mice either in lung or at any other sites (*230*). However one study reported the induction of lung tumors, mainly adenocarcinomas and squamous carcinomas, by the weekly intratracheal instillation of nickel powder (10 times 0.3 or 0.6 mg), oxide (10 times 5 mg or 15 mg), and nickel subsulfide (15 times 0.063 mg, 0.125 mg or 0.25 mg) in rats (*231*).

In inhalation experiments, low incidence of metastasizing malignant tumors were observed in rats after a single 30-min exposure to 0.25 mg/L or three weekly 30-min exposures to 0.03

mg/L nickel carbonyl for 1 year. As spontaneous malignant neoplasms in Wistar rats are rare, even the low incidence of pulmonary malignancies was of some significance. Exposure to 0.97 mg Ni/m³ as nickel subsulfide (70% of particles smaller than 1 μm) for 78 to 80 days (6 hr/day, 5 days/week) significantly increased the incidence of squamous metaplasias, adenomas, and adenocarcinomas in the lungs of rats (*214*). Inhalation studies with metallic nickel powder and NiO gave negative results with C57BL mice, Wistar rats, hamsters, and guinea pigs (*215*).

Nickel subsulfide potentiated the carcinogenic effects of benzo[*a*]pyrene and 20-methylcholanthrene, while manganese powder and calcium or magnesium acetate inhibited the tumorigenic effect of nickel subsulfide (*214,215*). When injected intramuscularly together with nickel subsulfide in the same suspension, magnesium carbonate (but not acetate) inhibited (*233*) and zinc salts slowed down (*233*) the carcinogenic process. The protective effect of carbonate may have been mediated through the involvement of natural killer and phagocytic cells (*232*).

In summary, those nickel compounds which proved to be unequivocally carcinogenic by the inhalation route were nickel subsulfide and nickel carbonyl. Nickel dust and insoluble or sparingly soluble nickel compounds, with few exceptions, caused injection-site sarcomas.

Kinetic Considerations

The gastrointestinal absorption of nickel is 1 to 5% in humans. In animals, 90% of the ingested dose was recovered in feces. Bivalent nickel penetrates the skin at sweat ducts and hair follicles and binds to keratin (*214,234*).

Nickel carbonyl freely crosses the alveolar membrane, and therefore its pulmonary absorption is rapid and extensive. Particles deposited in the conductive airways are cleared by mucociliary action into the alimentary tract. Clearance from the alveolar region is through absorption and/or the phagocytosis of particles by monocytes, macrophages, and polymorphonuclear leukocytes. For some compounds, the solubility is significantly higher *in vivo* than in water. Moreover the dissolution of particles is much faster within cells than extracellularly, probably because the phagocytized particles are exposed to the acidic pH of the vacuoles (*225,235*). There are no reliable data on the deposition of nickel in the respiratory tract of exposed workers.

In golden hamsters, 20% of the inhaled nickel oxide aerosols (particle size 1.0–2.5 μm) was deposited in the lungs. The pulmonary clearance half-time was approximately 45 days. Pulmonary deposition was inversely related to particle size after the long-term exposure of rats to NiO aerosol. The absence of increases in nickel concentrations in blood or soft tissues indicated negligible absorption. Similar results were obtained with nickel-enriched fly ash in hamsters. With welding fumes, the pulmonary clearance half time was 30 to 66 days. Contrary to NiO, the pulmonary elimination of nickel after the intratracheal instillation of $NiCl_2$ is rapid. In rats the pulmonary clearance half-time was 4.5 hr in one study and 70% of the dose cleared within 24 hr in another study. Other pulmonary clearance half-times after intratracheal instillation were 72 hr for nickel carbonate in mice and about 3 days for nickel subsulfide in rats (*214*). In mice, 40% of the intratracheal dose of nickel subsulfide reached the alimentary tract and was excreted in feces, and the remaining 60% was excreted in urine. Lung clearance had initial and final half-times of 1.2 and 12.4 days (*236*).

A comparative study showed that 1 day after the intratracheal instillation of 1 μmole Ni compounds to rats, the quantities of pulmonary nickel were 0.13 μmole from $NiCl_2$; 0.14 μmole from $NiSo_4$; 0.39 mole from Ni_2O–; and 0.65 μmole from NiO. In the next 6 days, only the two highly soluble compounds continued to clear rapidly (*237*). After 12 days (*238*) or 13 weeks (*239*) of inhalation exposures of rats or mice to equimolar concentrations of nickel compounds, $NiSO_4$ produced the lowest pulmonary deposition, followed by Ni_2O_3 and NiO. After the longer exposure period, the concentration ratios were 1:2.3:24 in rats and 1:14.2:51.8 in mice. Both pulmonary accumulation and exposure time before steady state were inversely related to solubility (*239*); the order of pulmonary toxicity and the order of water solubility were the same (*239,240*).

Inhalation (6 hr/day, 5 days/week) of 0.44 mg Ni/m³ as Ni_2S_3 for 12 days produced scattered pulmonary inflammation (*241*), and exposure to 0.1 mg Ni/m³ for 13 weeks caused alveolar macrophage hyperplasia in all and chronic active inflammation in some of the rats (*239*). As the weekly exposure schedule was the same, it is a point of interest to compare air concentrations in this study and the carcinogenic study of Ottolenghi et al. [see U.S. EPA (*214*)]. Exposure of rats to Ni_3S_2 at a concentration of 0.97 mg Ni/m³ for 78 to 80 weeks induced lung hyperplasia in 64%, adenomas in 7%, and malignancies in 7% of exposed rats (*214*).

Differences in the potential of different nickel species to cause injection-site malignancies initiated research on cellular uptake and predictors of carcinogenic potential. Sunderman (*224*) compared the carcinogenic potential of nickel compounds with *a*) their dissolution half-times in serum and renal cytosol; *b*) phagocytic index in rat alveolar macrophages; *c*) hematocrit of rats after intrarenal injection; and *d*) the nickel mass fraction. The best predictor of carcinogenicity was hematocrit. Carcinogenicity also correlated with mass fraction, but not with solubilities or the phagocytic index. Nevertheless, within one class of nickel compounds (e.g., sulfides), phagocytosis by alveolar macrophages, and even more by facultative phagocytes, correlated well with carcinogenic potential (*242*). Heck and Costa (*242*) found that potent carcinogenic particulates, like crystalline NiS, Ni_3S_2, and NiO, exhibit strong negative surface charge in distilled water and enter cultured fibroblast cells rapidly. Noncarcinogenic, amorphous NiS has a slightly positive surface charge and has a low phagocytic index, and ionic nickel inhibits phagocytosis. The outcome of *in vitro* tests depends not only on the potency of nickel compound to induce a certain effect, but also on the ability of cells to incorporate the nickel compound. Thus, when concanavalin A was used to induce interferons-α/β, crystalline NiS had an inhibitory effect in murine L-929 cells that actively phagocytized the particles, but not in rat spleen cells with low phagocytic activity (*244*).

Within 1 week, the intrarenally injected crystalline Ni_3S_2 particles were phagocytized by the mesangial cells of the glomerulus. Because the dissolution half-time of crystalline Ni_3S_2 is 24 days in tissues, Ni_3S_2 particles were not detectable 10 weeks after injection, though some Ni_3S_2 was converted to crystalline NiS, which has a long dissolution half-time in tissues (*235*). Phagocytized nickel particles are known to aggregate around the nuclear membrane, and the released ionic nickel can enter the

nucleus (*235*). Contrary to intracellular dissolution, extracellular dissolution does not supply sufficient ionic nickel for the induction of carcinomas. This is shown by the negative outcome of treatment when 15 doses of 4.4 μmole Ni/rat, as soluble $NiSO_4$, was injected intramuscularly on every second day (*245*). Thus, incorporation of particles by target cells and intracellular dissolution seem to be the decisive factors in carcinogenesis by nickel particles.

In summary, these studies indicate that the high toxicity and rapid clearance of soluble nickel compounds is unfavorable for carcinogenesis. The toxicity, solubility, and clearance of nickel subsulfide favor carcinogenicity by allowing the survival of animals with a relatively high tissue dose and the prolonged exposure of target cells to a relatively high concentration of nickel ions.

Short-Term Tests

In vitro assays have been comprehensively reviewed by Coogan et al. (*225*) and Sunderman (*246*). Nickel compounds are almost always negative in mutagenicity assays with *S. typhimurium* and *E. coli*. Thus, the fluctuation assay, which identified chromate, beryllium, and cadmium as mutagenic and arsenite, arsenate, and lead nitrate as nonmutagenic, was negative with nickel chloride or sulfate (*48*). The exception was λ prophage induction in *E. coli* which has a much wider genetic end point (*247*).

In mutagenicity tests employing Chinese hamster V-79 cells, Chinese hamster ovary cells, rat hepatocytes, human diploid foreskin fibroblasts, and murine lymphoma L51784Y cells, statistically significant mutagenic effects of nickel compnunds were seen only in the murine lymphoma assay (*246*). However, a strong mutagenic response was observed in rat kidney cells infected with mutant murine sarcoma virus DNA, which has a defect in the expression of the v-*mos* transforming gene. A brief incubation with subtoxic concentrations of $NiCl_2$ induced alterations in gene expression resulting in the heritable extension of temperature permissiveness of splicing from 33°C to 37°C (*248*). Similarly, a strong mutagenic response was induced by NiS in the g12 Chinese hamster cell line with a single copy of the bacterial *gpt* gene stably inserted into the V79 genome (*249*). Nickel ions also enhanced UV-induced mutagenesis in V-79 cells (*178,250*) and the mutagenic effect of methyl methansulfonate in polymerase-proficient *E. coli* and in *S. typhimurium* T101, provided there was a functional *recA* gene product (*251*). In HeLa cells, there was a good correlation between the co-mutagenicity of Ni(II) with UV light and the inhibition of DNA repair (*131,250*).

Mammalian transformation assays gave positive responses with carcinogenic nickel compounds and some soluble nickel compounds of unproven carcinogenicity. In Syrian hamster embryo cells, $NiCl_2$, $NiSO_4$, Ni_2O_3, nickel dust, Ni_3Se_2, crystalline NiS, and Ni_3S_2 produced morphological transformation. Amorphous NiS yielded negative response and NiO gave equivocal results. In agreement with the avid cellular uptake of crystalline NiS by phagocytosis and the moderate uptake of Ni^{2+} ions by ion channels, crystalline NiS was 2.5 times as potent as $NiCl_2$. Comparison of different nickel compounds indicated that the intracellular bioavailability of Ni^{2+} is the property that determines the transforming potency. The transforming ability of nickel compounds was confirmed in BHK-21 hamster kidney cells. Transformation was observed also in cell cultures derived from the progeny of Syrian hamsters. Mothers were treated with $NiSO_4$ on day 11 of gestation, and progeny was excised 2 days later. In contrast to strongly positive results in Syrian hamster embryo cells, human cell cultures were only partially susceptible to nickel. Thus, human fetal kidney explants exposed to $NiSO_4$ developed signs of transformation, but transformed colonies failed to develop tumors after implantation in nude mice. In human diploid foreskin fibroblasts, only anchorage independence developed, and none of the signs of transformation were present in bronchial epithelial cells exposed to $NiSO_4$. In transformation assays, nickel compounds acted synergistically with benzo[*a*]pyrene (*246*). The absence of mutation to ouabain resistance indicates that the Ni(II)-induced morphological transformation of 10T1/2 mouse embryo cells was not through base substitution mutation (*55*). Abnormal chromosomes, loss of normal chromosomes, and increased number of chromosomes were seen in human fetal kidney epithelial cells immortalized by nickel sulfate (*252*). The transformation of Syrian hamster embryo cells by $NiCl_2$ was inhibited by the presence of $MgCl_2$ (*253*).

Intraperitoneal administration of $NiCl_2$ to mice and hamsters caused chromosome breaks and gaps in bone marrow. No SCE was observed in the lymphocytes of active and retired refinery workers. However, in several *in vitro* systems (e.g., human lymphocytes, Syrian hamster embryo cells) nickel compounds increased not only chromosome breakage, but caused SCE frequently in a dose-related manner (*246*). Nickel ions preferentially damaged centromeres and other heterochromatic regions of CHO cell chromosomes. Elevation of magnesium chloride levels reduced the cellular uptake Ni^{2+} DNA strand breaks, and DNA-protein crosslinking, but did not reduce damage in the euchromatic regions (*254*). Deletion of the heterochromatic long arm of the X-chromosome was the only Ni-induced karyotypic alteration in male Chinese hamster cells. These have heterochromatin along the entire long arm of the X-chromosome and exhibit an unusually high incidence of nickel-induced transformation compared with cells from females of the same species. The potently mutagenic and carcinogenic chromates do not produce a predominance of SCEs in the heterochromatin region (*255,256*). In human lymphocyte culture, $NiSO_4$ reduced chromosomal length, indicating that Ni^{2+}—at least at high though not lethal concentrations—is a powerful spindle inhibitor (*257*).

The IP injection of nickel carbonate to rats caused DNA strand breaks and the formation of DNA–protein complexes in renal cell nuclei. DNA strand breaks were also observed in cultured CHO cells exposed *in vitro* to crystalline NiS, or N_3S_2, but not in cells exposed to amorphous NiS. However, strand breaks occurred only at concentrations that reduced cell survival (*246*). Exposure of either whole CHO cells or isolated nuclei to $NiCl_2$ increased in a dose-dependent manner the binding of satellite DNA to protein (*256*). Most of the proteins tightly bound to DNA were nonhistone chromosomal proteins (*259*). BALB/c-3T3 cells that survived exposure to increasing concentrations of $NiC._2$ retained resistance to Ni(II) for many generations. This resistance is associated with heterochromatic abnormalities involving fusions at the centromeres (*260*).

In vitro submillimolar concentrations of $NiCl_2$, $NiCO_3$, and crystalline Ni_3S_2 induced transition of double-stranded DNA

from right-handed to left-handed helical form. Other metals which have the same effect are cobalt and manganese ions. Removal of the responsible cations by a completing agent resulted in instantaneous reversal (*246*). The complex formation between Ni(II) and synthetic polydeoxynucleotides with alternating G-C sequences was associated with conformational changes. The Ni-oligopeptide complex was insensitive to DNAse-I digestion and participated in oxygen biochemistry with the postulated involvement of the Ni(III)/Ni(II) redox couple or the $(NiO)^{2+}$ oxene species (*261*). The Ni(III)/Ni(II) redox couple had a role in the *in vitro* polymerization of histones by Ni_3S_2 (*262*). The binding of Ni(II) to DNA and the subsequent formation of a nickel–oxygen complex was assumed to be the reactive species responsible for DNA damage (*178*). $NiCl_2$ impaired the fidelity of DNA synthesis in a cell-free system (*64,180*). Thus, though the nickel ion in most of the gene mutation assays gives negative or weakly positive responses, it has a high gentoxic potential in several other assays.

Conclusions

There is convincing evidence that nickel dust, present in the working environment, can cause cancer in the lung and the nose. There is some indication that cancer risk is attributable to oxidic and sulfidic nickel and that soluble nickel compounds enhance the carcinogenic risk of exposure to less soluble forms. In animal experiments, carcinogenicity depends on the route of administration. Injection-site tumors can be caused by nearly every insoluble or sparingly soluble nickel compound and also by nickel powder, while only nickel subsulfide was consistently carcinogenic after intratracheal instillation or inhalation. The moderate solubility of nickel subsulfide particles allows time for phagocytosis, and the aggregation of particles around the nuclear membrane where slowly released nickel ions can enter the nucleus and react mainly with heterochromatin. The carcinogenic dose is higher than the dose that produces chronic, active pulmonary inflammation.

The mutagenicity of Ni(II) in bacterial and mammalian cells ranged from negative to weakly positive. Strong positivity was obtained when viral or bacterial genes were introduced into a mammalian cell system. Although Ni(II) is at best a very weak point mutagen, it is strongly clastogenic, inducing chromosomal breaks, gaps, deletions, and SCE. DNA strand breaks, inhibition of excision repair, and morphological transformations are induced by Ni(II) in several mammalian cell systems. Ni(II) is also active in cell-free systems, as shown by increased binding of DNA to proteins, helical transformation, and impairment of the fidelity of DNA synthesis.

Though the causal role of these interactions in nickel carcinogenesis is unproven, they demonstrate the ability of nickel to induce genotoxic events. Sunderman (*246*), who in his review listed additional interactions (blocking cell-to-cell communication, stimulating lipid peroxidation, reacting with the finger-loop domains of transforming proteins), expressed the view that "a clear delineation of the cellular and molecular mechanisms of nickel carcinogenesis will be achieved by the year of 2000."

Miscellaneous Metals

Antimony

After the introduction of control measures in 1960, the average atmospheric concentration of antimony was nearly 20-fold the 0.5 mg/m³ TLV in an antimony smelter. It is most likely that exposure before 1960 was substantially higher. No excess deaths occurred among those employed after 1958, but the lung cancer mortality of workers employed before 1958 showed a 2-fold excess over the number expected from local mortality rates. Six of the fifteen who died of lung cancer were very heavy or heavy smokers, 5 were moderate smokers, one was a light smoker, and 2 were classified as nonsmokers (*32*). Arsenic in antimony ore as well as smoking might have been confounding factors.

Prolonged inhalation exposure (7 hr/day, 5 days/week, up to 1 year) to 36 to 45 mg Sb mg/m³ in the form of antimony trioxide (containing 80% Sb and 40 ppm As) or antimony ore (containing 46% Sb and 792 ppm As) induced lung tumors, including carcinomas, in 26% of female rats, but none in male rats. Pulmonary antimony concentrations were 1.5-fold higher in males than in females, while the concentration of arsenic was the same. In other tissues, arsenic concentrations were higher in females than in males. This suggests that if arsenic contributed to the carcinogenic process, the systemic concentration of arsenic was more important than the pulmonary concentration (*263*).

Several antimony compounds were negative in the Ames test and *rec* assay (*4*). In the *rec* assay with *B. subtilis*, Sb(VI) was strongly and Sb(III) weakly positive (*125*). Sb(III) enhanced the transformation of Syrian hamster embryo cells by simian adenovirus (*54*).

Cobalt

Increased lung cancer mortality was observed in Soviet cobalt recovery workers who were also exposed to arsenic and nickel (*2,5*). In animal experiments, cobalt induced injection-site malignancies (*4*). Multiple IP administration of cobalt(III) acetate did not increase the incidence of lung adenomas in strain A mice (*200*). Inhalation exposure to aerosols of CoO or intratracheally instilled cobalt dust was not tumorigenic in hamsters (*4*).

$CoCl_2$ was not mutagenic to Salmonella strains in plate incorporation and fluctuation assays (*48*) and it was also negative in the *E. coli* λ prophage assay (*247*). In the *rec* assay with *B. subtilis* $CoCl_2$ was slightly positive. In mouse lymphoma cells, cobalt(II) chloride was not mutagenic (*126*), and in Chinese hamster cells it was slightly mutagenic at very low rates of cell survival (*264*). Treatment with Co(II) resulted in the following *in vitro* changes in mammalian cells: increased SCE in human lympiocytes (*34*), moderate enhancement of the transforration of Syrian hamster embryo cells by simian adenovirus (*54*), DNA strand breaks (*34*) and increaed DNA-repair synthesis in cultured CHO cells (*209*). Co(II) increased misincorporation in the daughter strand of DNA in cell-free systems (*64,180*).

Copper

The intratesticular injection of $CuSO_4$ or $CuCl_2$ induced testicular tumors in mice and chicken. Intramuscularly or intraperitoneally administered copper powder, copper acetate, and

copper sulfide were not carcinogenic in the mouse or in the rat. The induction of injection-site tumors produced by Bordeaux mixture (a mixture of copper compounds and calcium arsenate) has been attributed to arsenic (*4*). Cu(III) was negative in the mouse lung adenoma test (*200*). Copper compounds suppressed the carcinogenicity of ethionine, dimethylnitosamine, dimethylbenz[*a*]anthracene, and the promoting effect of phorbol esthers (*4*).

$CuCl_2$ was not mutagenic to Salmonella strains in plate incorporation and fluctuation assays (*48*). It was also negative in the *E. coli* λ prophage and the Trp^+ reversion assay, but it enhanced UV-induced mutagenesis (*247*). Cu(II) was mutagenic in CHO cells (*34*) and caused a slight increase in DNA-repair synthesis (*209*). $CuCl_2$ moderately enhanced the transformation of Syrian hamster embryo cells by simian adenovirus (*54*) and increased misincorporation in the daughter strand of DNA (*64,180*).

Iron

Increased lung cancer mortality was observed in hematite miners in England, Sweden, France, and the U.S.S.R., but not in Minnesota. Exposure included radon and radon daughters (*2,5*). Some epidemiological studies suggest that high body iron stores increase the risk of cancer (*265,266*).

Injection-site malignancies were induced by the SC injection of iron-dextran (*4*). Iron(II) 2,4-pentanedione was negative in the mouse lung adenoma assay (*200*).

Inhalation exposure of Syrian hamsters to ferric oxide (Fe_2O_3) dust enhanced diethylnitrosamine tumorigenicity in the peripheral lung (*267*). Dietary iron (3.5% Fe-fumarate) enhanced dimethylhydrazine-induced colon carcinogenesis in mice (*268*). A single dose of Fe-dextran (600 mg Fe/kg) sensitized *Ah*-responsive C57BL/10ScSn mice to the induction of hepatocellular carcinoma by polychlorinated biphenyls (*269*). Pretreatment of mice with iron-dextran (two IP doses of 12.5 mg Fe/kg, 12 hr apart) enhanced the growth of L1210 tumor cells, which were inoculated into the peritoneum 6 hr after the second iron dose (*270*).

Mutation to 6-thioguanine resistance was induced in CHO cells by Fe(II). Neither Fe(II) nor Fe(III) induced SCE in Don Chinese hamster cells (*34*). Fe(II) caused no morphological transformation in Syrian hamster embryo cells (*53*) but enhanced transformation induced by simian adenovirus (*54*). The fidelity of DNA synthesis was not altered by Fe(II) (*180*).

Manganese

There are no reports indicating that manganese is a human carcinogen. In rats, manganese acetylacetone (suspended in trioctanoin), but not pure manganese powder or manganese dioxide, induced injection-site tumors (*2*). The prolonged IP administration of manganese sulfate increased the incidence of lung adenomas in strain A mice (*200*).

Manganese chloride or potassium permanganate gave negative response in several Salmonella strains in both incorporation and fluctuation assays (*48*) but was positive in the *E. coli* λ prophage assay (*247*). Manganese was mutagenic in yeast and bacteriophage T4. It has been suggested that manganese acts as an error-producing factor in yeast mitochondrial DNA through the replacement of the magnesium cation in DNA polymerase (*2*). Mn(II) induced resistance to 8-azaguanine in CHO cells. In human lymphocytes, Mn(II) produced an increase in the incidence of SCE (*34*).

In C3H mouse mammary carcinoma (FMSA) cells, 1 mM Mn(II) or Mn(VII) induced chromosomal breaks and exchanges in about 5% of cells. Above this concentration, Mn(VII) increased aberrations to a higher level, whereas the cytotoxic effects of Mn(II) precluded scoring (*271*). *In vivo* the cationic and anionic salts of manganese were clastogenic in mouse bone marrow. In relation to their respective LD_{50} doses, manganese chloride was a more potent clastogen than permanganate (*272*). However, the analysis of the data indicates that on a mole per kilogram basis, the two compounds were equally potent clastogens and that the total number of chromosomal aberrations was increased more by permanganate than by $MnCl_2$. The insoluble MnS may be more genotoxic than the soluble $MnCl_2$. Manganese sulfide, which is taken up by cells through phagocytosis, inhibited the growth of Chinese hamster V79 cells at less than 1/100 of the inhibitory dose of the soluble $MnCl_2$ (*247*). Mn(II) decreased the fidelity of DNA synthesis in cell-free systems (*180*).

Selenium

Epidemiological studies gave conflicting views on the carcinogenicity and anticarcinogenicity of selenium. Both the epidemiological and experimental evidence of selenium carcinogenesis have been reviewed by a WHO Task Group (*273*). The group did not accept the conclusions of five studies because of shortcomings in design or evaluation. The group also noted that selenite and selenate had induced hepatic malignancies only at exposure levels that produced liver cirrhosis. The sixth study observed no signs of neoplasia in mice given selenite in the drinking water (up to 8 mg Se/L) for 50 days, and in the seventh study, 15 mg/kg SeS given by gavage for 103 weeks (5 days per week) induced hepatocellular carcinomas in both male (29%) and female (42%) rats. When 100 mg SeS/kg was given in the same schedule to female mice, the incidence of cancer was the same as in female rats given 15 mg/kg. Male mice were resistant. Alveolar/bronchial carcinomas developed in 8% of female mice. Nevertheless, the WHO Task Group concluded that the available experimental data were insufficient to allow the classification of inorganic seleniumas a carcinogen.

It has been shown that selenium dioxide and sodium selenite, given in food or drinking water, have a preventive effect chemically induced and spontaneous, presumably virally induced, cancers. Generally, the level required for protection ranged from 1 to 6 mg/kg food or drinking water. Such a supplementation results in intakes that are considerably below intakes used in carcinogenesis studies, but they are considerably in excess of the animal's nutritional needs (*273*).

Selenate was mutagenic to Salmonella in the plate incorporation assay (*48*) and SeO_2 gave strong positive reaction in the *rec* assay with *B. subtilis* (*125*). In the *rec* assay, selenate was less potent than selenite, but in cultured human fibroblasts, Se(IV) and Se(VI) induced DNA repair to the same extent. Selenite increased DNA fragmentation in human fibroblasts and increased SCE in human lymphocytes (*273*). Selenite decreased the mutagenicity of several organic compounds and exerted an antagonistic effect against clastogens (*273*).

Zinc

There are no observations to suggest that zinc is a human carcinogen. On the contrary, there is some indirect epidemiological evidence that zinc deficiency may increase susceptibility to esophageal carcinogenesis (*4*). In animal experiments, only intratesticular administration induced cancer. When administered by this route, zinc chloride or zinc sulfate induced testicular teratomas in the fowl, rat, and hamster (*4*). Zinc acetate was negative in the mouse lung adenoma assay (*200*). The effects of zinc chromate or the complexes of zinc with dithiocarbamate derivatives (e.g., zineb) are independent of the presence zinc.

Zinc antagonized the carcinogenicity of cadmium (see section on cadmium) and some organic compounds. There is some evidence that zinc deficiency increases susceptibility to cancer, that small doses provide protection, and that prolonged excessive zinc intake promotes tumor growth (*4*). Dietary zinc deficiency (7 μg/g or less in the diet) suppressed the growth of transplanted tumor cells in DBA/2 mice. The effect of high dietary levels (>200 μg/g) was equivocal (*274*).

Zinc(II) was not mutagenic to *E. coli* (*166*). The *E. coli*λ prophage assay was also negative with zinc (*247*). Zinc(II) induced resistance to 8-azaguanine in CHO cells (*34*) but not to trifluorothymidine in mouse lymphoma cells (*126*). Zinc(II) did not induce morphological transformation (*53*) but moderately enhanced simian adenovirus-induced transformation in Syrian hamster embryo cells (*54*). There is no indication that zinc can cause chromosomal damage, DNA strand breaks, or that it can alter the fidelity of DNA synthesis (*55*).

Conclusions

Several of the metals discussed in this section influenced carcinogenesis, but only one metal, antimony, has been implicated as a carcinogen by both epidemiological and experimental studies. All the other seven metals are essential elements, and their influence on carcinogenesis ranges from protection to promotion. Most of these metals, with the exception of SeS, were able to cause only injection-site malignancies, and zinc was able to cause injection-site malignancy only after intratesticular injection. The outcome of short-term tests does not show a consistent pattern. Each of these metals was negative in some tests and positive in others.

Summary Conclusions

The first report on metal carcinogenesis, published in 1926, described testicular teratomas that developed after the intratesticular injection of zinc chioride to roosters (*2*). Though later the same response was observed in two other species, zinc was not even considered for carcinogenicity evaluation by IARC (*50*). According to one definition, a chemical is likely to be a human cancer risk if it is *a*) reliably active in a battery of short-term tests; *b*) definitively active (high yield of specific neoplasms, latent period less than 18 months) in several bioassay systems *in vivo*; and *c*) exhibits activity over a range of dose levels (*275*). While zinc definitely does not fit into this category, none of the metals satisfy all these requirements. It is usual that test results include one of more of the following findings: *a*) short-term tests are not uniformly negative or positive; *b*) tumors are induced only after a particular route of administration; *c*) the overlap between tumorigenic and toxic doses prevents the demonstration of tumorigenicity over a range of dose level; and *d*) only benign tumors are induced.

The use of short-term tests has some inherent difficulties. The Salmonella assay detected only about half of a sample of 44 rodent carcinogens and more than 30% of rodent noncarcinogens were positive in the assay (*276*). As usually a battery of tests is used, each test having a different sensitivity, the end result is a mixture of positives and negatives. The same metal may be nonmutagenic in the Salmonella test and mutagenic in *E. coli*; it may be negative in bacterial assays, but positive in mammalian gene mutation assays. Only one of the 10 metals, listed in Table 2, was unequivocally mutagenic in standard bacterial assays, though many were positive in the *E. coli* λ prophage assay, in bacteriophage T4, and in yeast, or in mammalian cells.

In addition to gene mutation assays, there are several other tests that measure in cultured mammalian cells biological changes (induction of cell tranformation, SCE, unscheduled DNA synthesis) related to, but without proving, mutagenicity and carcinogenicity (*276*). Nickel was nonmutagenic in mammalian cells, though it became mutagencic when the sensitivity of the system was increased with inserted viral or bacterial genes. Several metals were negative in morphological transformation tests but were able to enhance virally induced transformation. However, the most sensitive test may not be the best predictor of animal and human carcinogenicity. It is a reflection on doubts concerning the predictive value of genotoxicity tests that one of the three alternative solutions proposed by Ashby (*276*) is to "abandon the use of the available genotoxicity assays and to continue with the exclusive use of rodent carcinogenicity bioassays for the prediction of possible human carcinogen hazards." Others believe that at the present time both tests are needed because the strategy of extrapolation to human risk is different for genotoxic and nongenotoxic carcinogens (*277*). The classification of nongentoxic agents as potential human carcinogens requires understanding of the mechanism of action, reasons for target organ and species specificity, and the quantitative dose–response relationship between end points such as induced cell proliferation and carcinogenicity (*278*). In the field of metal carcinogenesis, the first obstacle for such a classification is that dose and route of administration often have no relevance to human exposure.

Though intratesticular administration did not mimic human exposure and local tumors developed at the site of parenteral administration are not a valid measure of carcinogenicity (*279*), bioassays for injection-site tumors have become popular models for the identification of carcinogenic metals and metal compounds. Table 2, which compares supporting evidence for the carcinogenicity of 10 metals, shows that the route of administration only rarely simulates route of human exposure. Compared with dietary, drinking water, or inhalation exposure, parenteral or intratracheal administration is precise, not labor intensive, and the treatment period is short (maybe only one injection). However, concentration at the site usually exceeds concentrations that can be achieved by oral or inhalatory dosing. The concentration of substance in a relatively small tissue volume enhances the possibility of severe injection-site damage. Finally, though a positive reaction identifies the test material as a

Table 2. Comparison of epidemiological evidence with experimental carcinogenicity and genotoxic reactions.

Metal	Epidemiology	Bioassays	Experimental: Gene mutation[a]	*rec* Assay	Chromosomal damage, SCE	Cell transformation[b]	DNA fidelity
As	Lung and skin carcinomas	Lung adenoma by intratracheal administration	−(b), ±(m)	+	+	i	−
Be	?	Osteosarcoma by IV, lung cc by inhalation or intratracheal administration	−(b), +(m)	+	+	i	+
Cd	Lung cancer (confounders?)	Lung cancer by inhalation (only in rats)	±(b), +(m)	+	Inconsistent	i	+
Co	No proof	Sarcoma at IM injection site	−(b), ±(m)	+	+	e	+
Cr	Lung cancer	Sarcoma at IM injection site, lung cc by implanted pellets	+(b), +(m)	+	+	i	+
Ni	Lung and nasal carcinomas	Local malignancies at various injection sites; lung cc by inhalation of Ni-subsulfide	−(b), −(m)	−	+	i	+
Mn	No proof	Lung adenoma by intraperitoneal administration	−(b), +(m)	−	+	e	+
Pb	Inadequate	SC: renal carcinoma; feeding; renal adenoma; IP; lung adenoma	−(b), ±(m)	−	Inconsistent	e	+
Sb	Lung cancer	Lung cancer by inhalation (only in female rats)	?	+	+	e	?
Zn	None	Testicular teratoma by testicular injection	−(b), ±(m)	?	?	e	−

[a](b) bacterial; (m) mammalian cells.
[b]i, inducer; e, enhancer.

potential carcinogen, the carcinogenicity of the metal may be restricted to the injection site. This was the case with zinc chloride.

The interpretation of benign tumors does not present problems when their incidence is increased with the incidence of malignant tumors. When there are only benign tumors, they have only a limited value as indicators of carcinogenic potential (*280*), and, in the hierarchy of evidence, adenomas alone have less value than the presence of malignant tumors. In addition to the general problem of benign versus malignant tumors, the value of the mouse lung adenoma assay is questionable on two grounds. First, animals that develop background tumors (e. g., lung adenoma in strain A mice) in high yield possibly do so in ways that are not relevant to other species or strains (*281*). Second, the predictive value of the assay for metals is low; it was positive for nickel, manganese, molybdenum, and negative for arsenic, cadmium, chromium.

A more general problem is the toxicity of metals at carcinogenic dose levels. Thus, the immediate consequence of zinc chloride injected into the testes was necrosis followed by chronic inflammation. Such circumstances raise the possibility that high-dose toxicity was responsible for cancer initiation. Cellular proliferation provides a favorable milieu for several stages of carcinogenesis (*278,231*). Local trauma followed by cellular proliferation is a common observation in carcinogenicity studies with metals. Whatever the route of administration, the carcinogenic dose of a metal always inflicted nongenotoxic damage on the carcinogenic target. Thus, kidney tumors developed at very toxic doses of lead and generally only in animals that experienced obvious acute lead toxicity, including kidney injury. The prelude to the induction of pulmonary carcinoma by beryllium, chromium, and nickel was always cell death and chronic inflammation. For solving the problem, Roe (*282*) suggested that tests for carcinogenicity be carried out at realistic levels of exposure or just above. Moreover, DNA abnormalities can be incurred by more subtle reactions than cell death and chronic inflammation. Thus, without any exposure to a carcinogen, significantly more tumors, including malignancies, developed in rats fed *ad libitum* than in rats given 20% less food (*282*).

The local damage caused by the testicular injection of zinc chloride indicated its dissociation to hydrochloric acid and biochemically active Zn^{2+}. As Zn^{2+} inhibits and stimulates several enzymatic reactions and it is present in RNA polymerases (*283*) and in the finger-loop domains in DNA-binding proteins that are regulators of gene expression (*284*), it is possible that HCl, as the primary cause of local trauma and cellular proliferation, and Zn^{2+} shared the responsibility for the induction of cancer. It is an attractive hypothesis that the ultimate carcinogenic form of a metal is its ion in a particular oxidation state, e. g., Ni^{2+}, Cr^{3+}, or Cd^{2+}, which must be delivered to or formed in the target cells. The proof of this ionic hypothesis would be the identification of those chemical reactions that are responsible for carcinogenesis and the participation of the ion in the crucial reaction. At present such an identification is not possible, mainly because of gaps in the knowledge of the cellular and molecular mechanism of metal carcinogenesis. Moreover, the single-ion hypothesis is challenged by observations that indicate that some of the genotoxic reactions are catalyzed by a redox pair [Ni(III)/Ni(II)] or an unstable intermediate oxidation state (Cr(V)].

From the practical point of view the main question is not so much the exact definition of the ultimate carcinogen but the relationship between the chemical and physical forms of metals at entry into the body and carcinogenesis. Differences in dissolution, transport though membranes, phagocytosis, change in oxidation state, and the site of this change contribute to this relationship.

The influence of these background factors change from one metal to another. In the case of chromium the most likely ultimate carcinogen is Cr^{3+} formed within the cell, though the contribution of an intermediary oxidation state [Cr(V)] can not be excluded. As Cr(III) is unable to cross cell membranes, the extracellular

reduction of Cr(VI) to Cr(III) is a detoxification process, and exposure to Cr(III) does not share the carcinogenic potential of exposure to Cr(VI).

For arsenic, the division of roles between As(V) and As(III) is less distinct, but as pentavalent arsenic is rapidly converted to trivalent arsenic and the clastogencic potential of As(III) is severalfold the clastogenic potential of As(V), the active species is most likely AsO_3^{3-}.

Beryllium and cadmium exists only in the bivalent oxidation state, whereas lead and nickel exist predominantly in the bivalent oxidation state. The available data are insufficient for a comparative evaluation of different lead compounds, but considering that lead can replace calcium at functionally important binding sites, it is most likely that the biochemically effective species of lead is Pb^{2+}. Though inhalation experiments with aerosols of cadmium did not show noticeable differences between the carcinogenicities of $CdCl_2$, CdO, and CdS, the metallothione-bound and cytosolic fractions of cadmium in lung were much less in CdS-exposed than in $CdCl_2$- or CdO-exposed rats. Thus, it seems that these fractions are not a reliable measure of the bioavailability of cadmium for the carcinogenic process because cadmium in other fractions may also be important contributory factors. Compared with CdO or $CdCl_2$, after exposure to CdS rats had a higher proportion of pulmonary cadmium in the noncytosolic fractions (cell debris, nuclear, mitochondrial microsomal components) where, at least part of cadmium might be Cd^{2+} bound to structural macromolecules. CdS particles might also contributed to the carcinogenic process.

Because soluble beryllium compounds precipitate in extracellular fluids, phagocytosis is the important mechanism of cellular uptake and dissolution within cells is the key condition for carcinogenicity. Phagocytosis and cellular dissolution are also important for all nickel compounds with the exception of nickel carbonyl. Membranes are no barriers against the lipophilic and volatile nickel carbonyl, but the inefficient membrane transport of Ni^{2+} dissociated from soluble nickel compounds allows time for clearance and prevents genotoxic damage. Conditions are particularly favorable for Ni_3S_2, which has a dissolution half-time of 34 days in serum and 21 days in cytosol, and its phagocytosis by facultative phagocytes is 7-fold higher than phagocytosis of the noncarcinogenic, amorphous NiS. However, phagocytosis and dissolution is not the only determinant of carcinogenicity. The carcinogenic crystalline NiS has an 8-fold higher phagocytic index and 29-fold lower cytosolic dissolution rate than amorphous NiS. Thus, when cells are surrounded by equivalent concentrations of crystalline and amorphous NiS, the intracellular release of of Ni^{2+} should be 3.6 times more from the amorphous form than from the crystalline form. Nevertheless, only the crystalline form induced morphological transformation.

Differences in the pharmacokinetics of metals from one species to another in administration routes, dose, and exposure schedule may contribute to differences in the carcinogenic reponse of test animals and exposed human populations. However, carcinogenic exposure to metals is accompanied by a common reaction in both humans and experimental animals. Epidemiological studies have confirmed that metals increased cancer incidence when exposure was in the toxic range. The increased incidence of lung cancer in arsenic-exposed populations was regularly associated with lesions of skin and mucous membranes. Proteinuria in cadmium-exposed workers, ulceration of the nasal septum in workers exposed to chromium, and chronic respiratory irritation in nickel-exposed workers were frequently recorded in workplaces where there was an excess of mortality from respiratory cancer. The presence of nongenotoxic effects in both exposed populations and experimental animals and also negative or low mutagenicity in Salmonella and mammalian cell assays suggest that metals are with the possible exception of chromium, promoters rather than initiators and therefore their carcinogenicity depends on the threshold of toxicity. The nongenotoxic mechanism may explain why animal experiments gave some evidence for the carcinogenicity of beryllium, lead, cobalt, manganese, and zinc, and epidemiological studies were either ambiguous or negative. In agreement with this view, epidemiological studies, especially the sequence of follow-up studies indicate that improvement in the control of occupational exposure to arsenic, cadmium, and nickel was uniformly followed by a decrease in SMRs, often to the control level.

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