Environmental Health Perspectives
Vol. 40, pp. 83-91, 1981

Bioassay of Metals For Carcinogenesis: Whole Animals

by Arthur Furst*

Metals have been evaluated as potential carcinogens by administering pure elements or compounds by a large variety of routes. These include mixing the agent in the food, dissolving the test compound in the drinking water, or administering the material by gavage. The respiratory tract routes tested include inhalation, intratracheal instillation, the direct injection of particulates into the pleural cavity, or the implantation of hooks by surgical intervention. The parenteral routes used were intravenous injection, intraperitoneal injection, subcutaneous implantation, as well as intrafemoral and intramuscular injection. This latter route is the most commonly used. There are major objections to the subcutaneous implantations route, and data generated from these experiments are difficult to interpret for the foreign body reaction may give rise also to fibrosarcomas. This then is a nonspecific reaction.

Exotic routes tested include intrarenal, intratesticular, and intracranial injections. The endpoints of the carcinogenic reactions are, in the main, sarcomas of certain types with fibrosarcomas predominating. Rhabdomyosarcomas are the next most frequent cancer found, and squamous cell carcinoma may account for less than 2% of the cancers reported. Much more research is necessary to clarify the nature of metal carcinogenesis. Dose-response information is almost nonexistent; the divided dose problem has not been studied adequately, and very little information is available on interspecies reactions. More work is needed to help interpret the mechanism of action.

Introduction

Metals as a class are often neglected in reviews and compilations on carcinogens. Yet metals are much more important than most of the academically interesting carcinogenic agents; the use of metals in manufacture is increasing, and larger segments of the population are being exposed to metals in both the industrial setting and in the environment. When metals are mentioned in publications, superficial statements are often made, such as, "Recent studies have shown that nickel oxides and sulfides are capable of producing muscle tumors in rodents" (*1*). What is wrong with this statement is that nickel oxide has been shown active only by the intracranial route, and there are two sulfides of nickel: the ore, Ni_3S_2 which is active, and the more common nickelous sulfide, NIS which is inactive (*2*).

Perhaps, because of the extensive reviews by Sunderman Jr. (*2-5*) nickel has been mentioned most often as an example of a metal carcinogen. The first general review of metal carcinogens appeared in a book by Furst (*6*); subsequent reviews were published by Furst and his colleagues (*7-11*). The International Agency for Research on Cancer (IARC) of the World Health Organization has published two monographs on metals as possible risks to man (*12,13*) and a third appeared recently (*14*).

Uniqueness of Metals as Carcinogens

Metals are different from all other carcinogens. This may be because so little is known about the mechanism of action of this class of agents. Unlike the vast majority of known organic carcinogens, this class appears not to require activation; thus the ion or an ion complex appears to be the ultimate carcinogen. It is still possible that an unusual

*Institute of Chemical Biology, University of San Francisco, San Francisco, California 94117.

oxidation state of the carcinogenic element is involved. Unknown as yet are three important aspects of action: how the element or ion penetrates the cell membrane; how metals combine with nucleic acids, once within the cell, and hence distort the genetic information transmitted from cell to cell; and how a cell recognizes the difference between a carcinogenic ion, like Ni(II) and the noncarcinogenic ion (Cu(II).

Generic Classification of Individual Compounds

Should elements be classified in a generic sense, and the term carcinogenic metal or carcinogenic ion be used? Almost all, but not all nickel compounds induce fibrosarcomas when injected intramuscularly in the leg of rats (*4,15*). For example, nickel sulfide is not active (*4*), nor is nickel chloride (*16*). An organic compound of manganese was found to be carcinogenic, but not the free element nor manganese dioxide (*17*).

It is not possible at the present time to draw final conclusions on the basis of the available data to determine if an element is to be classified as a "carcinogenic element" when a single compound of that element induces a cancer in a valid experiment.

Foreign Body Carcinogenesis

What complicates this field is that rodents react to subcutaneously or intraperitoneally implanted solids or viscous liquids in a special way. In the vast majority of the cases these rodents develop fibrosarcomas at the site of implantation. The physical-chemical properties of the implanted solid determine the biological reaction, and a smooth surface seems to be one major requirement (*18*). Northdurft (*19*) first showed discs implanted subcutaneously will produce this phenomenon. One year later Oppenheimer et al. (*20*) implanted a number of foils subcutaneously in rats and indeed did obtain fibrosarcomas at the site of the implantation. This is often referred to as the Oppenheimer Effect. Oppenheimer also induced this type of tumor with plastic sheets (*20*). Subsequently Northdurft (*21*) found that the tumor yield and lag time before the appearance of the tumor depended upon what part of the anatomical site was implanted with the solid.

Foreign body carcinogenesis is distinct from metal carcinogenesis. This field, using silicates as examples, has been discussed by Bryson and Bischoff (*22*), and the general topic has been reviewed by Brand (*23*). Any experiment which uses these two routes, intraperitoneal and subcutaneous, to implant metals or insoluble compounds must be considered invalid.

Routes of Administration

Practically every conceivable route of administration has been used to test pure metals or selected compounds for their potential carcinogenic property. A summary of these routes will be given in this section. From most of the information found in the literature it is very difficult or even impossible in most cases to relate the relevancy of the results of these experiments to humans. Realistically, humans are exposed to metals or compounds in the work place, as environmental pollutants in the air, or in the drinking water. Hence, in the occupational setting the real world exposure routes are skin contact or inhalation; in the environment, other humans take in metals by inhalation or by the oral route from the contaminated water, or from food. With the exception of accidents, parenteral routes of administration are not realistic for the vast majority of humans.

Now, metal implants must be considered a route of entry of metals into the human body, for more and more prosthetic devices made of alloys, in contrast to plastics, are being used less so now than in the past. Caution must be used in the selection of the right alloy for prostheses, such as hip replacements; not only must the physical characteristics be considered, but also the metal-tissue interaction (*24*).

Many examples will be given of experiments where different routes of administration have been used; a truly exhaustive literature compilation is beyond the scope of this review.

Oral Routes

Very few experiments have been carried out using the oral route for chronic administration of inorganic substances, and with very few exceptions, no tumors have been reported when laboratory animals have been given the test compounds orally.

Gavage. One publication was found which reported the administration of a pure metal. Furst et al. (*25*) gave lead powder suspended in corn oil by stomach tube to Fischer-344 rats; no excess tumors were found. After dosing CD hooded rats with cadmium chloride by stomach tube, Levy and Clack (*26*) reported no tumors in two years.

Diet. This route of administration for metals or their compounds has seldom been used. To date, when compounds of metals have been mixed with

the food and fed to experimental animals, only with lead have tumors been reported. Among the published experiments are: nickel sulfate given to Wistar rats or dogs (*27*), bismuth oxychloride to BD rats (*28*), lead arsenate or sodium arsenate to Wistar rats (*29*), and two tin compounds fed to rats (*30*). Boyland et al. (*31*) fed lead acetate at a level of 1% in the diet to Wistar rats and found that 16 out of 20 surviving rats developed renal carcinomas. Van Esch et al. (*32*) found similar tumors also in Wistar rats fed basic lead acetate. Van Esch and Kroes (*33*) fed the same compound to Swiss mice in the diet and again, reported that renal tumors developed.

Water. Most of the research on treating rats and mice orally was conducted by dissolving the inorganic compounds in distilled water and giving these solutions to the test animals as their drinking water.

Perhaps the majority of the experiments in this field were carried out by Schroeder and his colleagues. They dissolved a number of compounds at levels to which they postulated humans were exposed, and gave these solutions to their test rats or mice. Among the compounds evaluated were: scandium, chromium(VI), gallium, yttrium, rhodium, palladium, and indium in mice (*34*); rhodium and palladium exhibited a slight carcinogenic action. Selenate but not tellurite was active in rats (*35*). By this procedure none of these inorganic ions was found to be tumorigenic in mice: chromium, lead, cadmium, nickel, and titanium (*36*), nor were mercury, methylmercury, nor aluminum (*37*). Schroeder summarized all of his work in a chapter entitled "Recondite Toxicity of Trace Elements" (*38*).

It must be understood that Schroeder used dose ranges of 2-5 ppm (which was his estimate of the human exposure).

Respiratory Tract Routes

Inhalation Route. The most logical route of administration of powders and dusts of metals is by inhalation techniques. Results from these experiments can best be related to the human experience. Not too many reports are available using this route, and the earlier publications did not address the difficulties and the complications of inhalation experiments. A good deal is now known about which parameters need to be measured; the distribution of sizes and the particle size range are of paramount importance (*39*). These data are needed to make decisions regarding how much and where in the respiratory tract the particles are lodged. Information on the deposition of particles in the respiratory tract in both the human and experimental animals is well documented and is covered in a recent review (*40*).

Metallic nickel powder (no data were given on particle size) was tested by Hueper (*41*), who exposed both rats and guinea pigs. Both species did develop lung tumors. This is an unusual finding, for guinea pigs are among the most resistant of species to the induction of cancer by chemicals. Saknyn and Blokhin (*42*) reported that 1/26 rats exposed to dust containing nickel, a product used for the production of nickel-black, developed a squamous cell carcinoma without keratinization. Wehner (*43*), on the other hand, exposed hamsters over their life span to nickel oxide and found no carcinogenic response. An inhalation experiment with nickel sulfide* resulted in the exposed Fischer-344 rats developing various types of lung tumors (*44*).

No tumors were found when rats were exposed to ammonium bichromate (*45*). Beryl did induce some lung tumors in rats, but not in monkeys; squamous cell carcinoma was not found (*46*). Sanders et al. (*47*) exposed rats, nose only, to high-fired oxides of beryllium and found only 1/184 rats developed a lung tumor in the two-year exposure period.

Implantation and Intrathoracic. In an attempt to evaluate calcium chromate as a lung carcinogen, Laskin et al. (*48*) surgically implanted small metallic hooks in the lungs of rats; attached to these hooks were threads soaked in calcium chromate. Furst et al. (*49*) induced mesotheliomas in Fisher 344 rats by the intrathoracic injection of a suspension of powdered cadmium. Bischoff and Bryson (*50*) did similar studies injecting tin needles into mice lungs; no tumors were found. All of these techniques are traumatic.

Intratracheal Methods. Certainly inhalation mimics the human experience best when exposures to powders, dusts, and aerosols are to be considered. Practically, it may not be possible to do exposures by the inhalation route for a number of reasons, although aerosols have been recently tested routinely (*51*). If the metal can not be suspended in air, the second choice will be the intratracheal instillation technique. This procedure becomes the first choice, if it is necessary to deposit on the lung surface a quantitative amount of the compound.

The most extensive use of this technique has been with chromates instilled into various strains of rats. An IARC monograph recently published (*14*) reviews these studies and evaluates the results.

*The authors call their compound nickel sulfide. Although this term is used in the trade, the actual compound used was apparently the nickel subsulfide. There is some confusion in the abstract literature concerning the chemical tested.

Hamsters have been the animal of choice when hydrocarbons have been tested for their carcinogenic properties relative to the respiratory tract. During the course of the hydrocarbon studies, ferric oxide was used as a carrier; this compound had to be tested as a control without the adsorbed benzo(a) pyrene (*52*). None of the control animals treated with the metallic compound, ferric oxide, developed lung cancers.

Ho and Furst (*53*) adopted this intratracheal instillation technique for use on mice, and demonstrated that the ferric oxide is widely distributed in the lungs of the instilled mice; the compound was not completely cleared even after two years. No neoplasms were found in the mouse lungs treated with ferric oxide in life-time studies.

Kasprzak et al. (*54*) instilled nickel subsulfide alone into rat lungs and after a period of time no tumors were reported; the combination, however, of this subsulfide and benzo(a)pyrene did produce lung cancer. It must be recognized that the nickel compound may have acted only as a carrier.

Tracheal Implants. Among the newer techniques developed to test the carcinogenicity of compounds is the use of tracheal grafts. The trachea of a test animal is carefully removed after sacrificing the animal and so cut that discrete rings are formed. A suspension of the metal compound under test is poured into the ring and permitted to harden; at that point the rings are transplanted subcutaneously into isogenic recipients; about two to six tracheas per rat are implanted. After a specific time the tracheas of the rats are removed and examined.

Using this technique, Lane and Mass (*55*) tested chromium carbonyl and found that 1/6 and 1/3 tracheas had squamous cell carcinomas; five of the Wistar Lewis rats with 2-5 tracheas each did not develop tumors. The experiments lasted 12-14 months. Tsutomu and Nettesheim (*56*) implanted two tracheal rings per female Fischer 344 rat, each graft containing 1 or 3 mg of nickel subsulfide. The experiments were carried out up to 20 months. Only 10% of the grafts were found with tumors; 67% of these were fibrosarcomas, and 1.3% were carcinomas.

Topical Application

Since humans are exposed to metal compounds by skin contact, especially in the work place, it is logical to study some suspect compounds for their activity by skin painting. Practically no publications are available where this route has been adequately tested using metal compounds. Both Khachatrian (*57*) and Kovalenko et al. (*45*) reported skin painting with chromium compounds; neither reported that tumors developed.

Parenteral Routes

The relevancy of data obtained by injecting metal compounds into test animals as applied to humans is questionable. Yet for convenience, and to answer academic questions, these routes are the most often used. It is important to know that the subcutaneous implantation route gives rise to site-implanted fibrosarcomas which may even metastasize to the lungs. However, because of the nonspecific aspect of this route, no extrapolation to human should ever be attempted when the only test route is subcutaneous for solids or viscous liquids.

In our laboratories we are finding another nonspecific pathological reaction. A few metals when implanted intramuscularly seem to induce osteosarcomas which also metastasize to the lungs. It does appear that this phenomenon should be considered an artifact of the experimental technique, rather than a true carcinogenic reaction to be related to the metal implant.

Intravenous Injection. Beryllium compounds have been given to rabbits by the intravenous route, and osteogenic sarcomas have developed (*58*); this route has been used by Schmahl and Steinhoff (*59*) to inject colloidal silver weekly into rats; a few tumors were found. Colloidal gold failed to induce tumors. Sunderman's group (*60*) gave six intravenous injections of nickel carbonyl (0.9 mg Ni/100 g) to Sprague-Dawley rats and found a variety of tumors; significantly, no squamous carcinoma of the lungs was reported.

Intraperitoneal Injection. The intraperitoneal injection of solids, dusts, or sparingly soluble metals, especially solids with smooth surfaces, in all probability will give rise to the nonspecific foreign body response, and in almost all cases will lead to the formation of fibrosarcomas at the site of injection. The reaction of mercury droplets injected intraperitoneally may be just this smooth surface effect (*61*). Soluble compounds which give rise to fibrosarcomas at the site may also not be true metal carcinogens.

Perhaps a different interpretation is needed when soluble compounds induce tumors at a site distant from the point of application. Gold thioglucose, when injected into female C57B1 mice produced obesity, and later the animals developed reticulosarcomas; there was no change in the expected spontaneous incidence of tumors (*62*).

Shimkin's group (*63*) uses the intraperitoneal

route to inject solutions of compounds to determine if they modify the incidence of the spontaneous pulmonary adenomas in Strain A mice. Before this method can be adopted as a screening procedure it must be validated by the testing of many more compounds, and the results compared with information obtained by other routes.

Subcutaneous Implant. The one route which should not be used to evaluate metals is subcutaneous implantation. Since the findings of Oppenheimer (*20*) and of Northdurft (*19*) that almost any implanted solid may induce a fibrosarcoma at the site, it is difficult to interpret the carcinogenicity of vitallium reported by Maltoni (*64*). Before conclusions can be drawn, this compound must be checked by another route, such as intramuscular. Doubt must also be cast on the report that rare earths such as gadolinium and ytterbium are carcinogenic because they induced fibrosarcomas following the subcutaneous implant of pellets into mice (*65*).

Similar to the remarks made under intraperitoneal routes, the appearance of tumors at the site after the subcutaneous injection of some soluble compounds may not be an indication of a true metal carcinogen. Cobalt chloride (*66*) and cadmium chloride (*67*) did induce local fibrosarcomas. In neither study did the authors determine if the compound precipitated at the site and thus became an implanted solid.

Intramuscular Injection. The most commonly used route to evaluate metals and their insoluble compounds as potential carcinogens is the intramuscular route. Usually powders are suspended in inert vehicles and injected deep into the thigh muscle of the rodent. Following the investigations of Gilman and Ruckerbauer (*68*), who found nickel subsulfide an active carcinogen using the intramuscular route, many others have used this procedure to test other metals and their compounds. Nickel subsulfide has been thoroughly tested by Sunderman, and the information is summarized in his elegant review (*4*).

Unlike either the subcutaneous or intraperitoneal routes, the intramuscular route can be used to differentiate between active and inactive compounds, at least under the conditions of these experiments. In the original experiment where nickel subsulfide was found carcinogenic (*68*), nickel sulfate was inactive, as were ferrous sulfide and copper sulfide. Furst and Schlauder (*69*) were able to distinguish between the carcinogenic activity of cadmium powder and the nonactive silver and gold powders. Many examples can be given, and comparisons of different compounds of the same element can be made; for instance both the oxide and the sulfide of cadmium induced tumors (*70*). Until contrary information becomes available, the intramuscular route would be the route of choice for the evaluation of metals and their sparingly soluble compounds; the experimental conditions to be met are that the fine powders be suspended in an inert vehicle, and that no discs be tested. However, it must be recognized that the relevance of this route to the human situation cannot be evaluated at the present time.

Intrafemoral Injection. In his pioneering work on metal carcinogenesis, Hueper (*71*) suspended nickel powder in a fatty vehicle and injected the mixture into a previously drilled hole in the femur. Fibrosarcomas resulted from this treatment. He was one of the very few to use this route. More recently, Mazabraud (*72*) injected zinc beryllium silicate into the tibial or femoral epiphysis and produced osteosarcomas in 70% of his treated rabbits. Since other routes can give similar results, this route is not recommended.

Miscellaneous Routes

Metals and their compounds have also been evaluated as potential carcinogenic agents by at least three unusual routes of administration. Although these routes were exotic, not all compounds tested by these routes of administration were found to induce tumors.

Intratesticular Injection. Perhaps the very first indication that an inorganic compound could induce a tumor in an animal was the work of Michalowsky (*73*), who in 1926 injected zinc chloride in the testes of roosters and later found teratomas. This experiment was repeated by Falin and Gromtseve (*74*) with zinc sulfate. By this route, copper sulfate was also found active (*75*). Tumors of the testes can also be induced in rats with zinc chloride (*76*) and by the implantation of nickel subsulfide (*77*).

Intrarenal Injection. Nickel subsulfide was injected intrarenally by a surgical technique into Sprague-Dawley female rats (*78*). Between 8 and 12 months, 40% of the test animals developed renal carcinomas which tended to evolve toward an anaplastic, spindle-cell variant. This compound was the only one which did induce the tumor; negative results were obtained with pure metal powders of nickel, cobalt, chromium, cadmium, lead, and gold. Cobalt sulfide, also tested, failed to induce tumors.

Intracranial Implantation. One of the very few routes by which nickel oxide induced cancer was by intracranial implantation (*79*). After a period of time, both sarcomas and meningiomas were found at the site of implantation.

Endpoints Found

A select group of pure metals, and some of their inorganic or organic compounds, are among the most potent of the chemical carcinogens. These agents have definitely induced malignant tumors in experimental animals; they fulfill the most stringent criteria which would determine whether a compound can be considered a true carcinogen (*7*). The time of appearance and the percentage of animals with tumors depends on the nature of the individual compound. Not enough dose relationships have been made to draw conclusions on this aspect of carcinogenesis.

Induced Malignancies

The vast majority of the tumors reported to be induced by metals and their compounds are classified as sarcomas. Fibrosarcomas predominate; these are invariably found at the site of implantation. The degrees of malignancy also vary. Both well and poorly differentiated fibrosarcomas have been described. Some years ago it was suggested that nickel was specific for the induction of rhabdomyosarcomas only; now it is recognized that compounds of nickel can induce leiomyosarcomas, fibromyosarcomas, fibromyxosarcomas, as well as sarcomas of uncertain types.

As noted, when beryllium compounds are administered intravenously, osteosarcomas appear. This author now believes that these tumors may be an artifact when solid inorganic compounds are injected intramuscularly too near a bone.

Very few carcinomas have been induced when metals and their compounds have been evaluated for their potential carcinogenicity. This tumor type was found in the intrarenal injection experiments, and a few were found in the treated tracheal grafts. Nickel carbonyl gas delivered to rats by the inhalation route was reported to induce squamous cell carcinoma of the lungs; this compound given intravenously, however, did not induce this cancer in the treated rats.

Nonmalignant Lesions

Injected metal powders have produced a variety of pathological conditions in the treated animals. The injected sites have at times become necrotic, leading to a variety of infections. Adverse reactions have been often mistaken for growths. Hemangiomas have also been seen.

Once in a while, a specific reaction is noted, such as the atrophy of the testes in the male rats treated with cadmium powder. An entire review is necessary for the description of the nonmalignant lesions found in the metal-treated animals.

Public Health Implications

Metals as such, or in alloys or catalysts, or as components in the electronic industries, and finally as components in inorganic and organic compounds, are being introduced into our civilization at a rapid rate. The general population is being exposed to greater quantities of these chemicals; the greatest health problem exists in the worker who is involved in the manufacture of metals or in the use of these materials.

Cancer does seem to appear more frequently among groups of workers who are exposed as copper miners, copper smelters (*80*) nickel refiners, or those who work with chromates (*81*). Sunderman (*2*) and Furst and Radding (*14*) have summarized the evidence for the nickel workers. It appears that the rates of cancer in this latter group are decreasing. Chromates have been implicated for a very long time as a metal carcinogen. The 1980 IARC review (*14*) has an overview of the entire chromate picture.

In addition to industrial exposure humans can be in contact with metals from the drinking water, and the polluted air. Some authors have attempted to correlate the concentration of carcinogenic trace metals in water supplies with cancer mortality of selected populations (*82*). (It is of interest that government agencies seem to give more attention to the organic compounds in drinking water than to its metal content.)

Gaps in Knowledge

Metal carcinogenesis, perhaps the least studied of any major class of active agents, requires further exploration.

Obviously the mechanism of action is not known, and the nature of the metal–nucleic acid interaction must be determined. Even before this important work, there must come some knowledge of the interaction of metal ions with proteins and small peptides of biological fluids. These studies may shed some light on how the ion crosses the cell membrane and actually gets into the cell to interact, if it does, with the intracellular DNA. Unknown also is how the nucleic acids are changed after the carcinogenic metal interacts with the normal nucleic acid. Finally, there is no information whatsoever to tell how a cell differentiates between a carcinogenic ion or compound and an inactive one. Thus there is insufficient knowledge for predicting which metals will be carcinogenic.

Recommended Research

In order to understand more about metal carcinogenesis, more research should be conducted both *in vivo* and *in vitro* along these lines.

1. Dose-Response. Too few studies have been conducted to relate the effect of the dose level given to animals and the tumor yield. The lag time before tumors appear must also be further investigated. (At least by the intramuscular route it is possible to palpate early tumors at the site of injection without sacrificing the animals. By use of calipers it is possible to get rate of increase of tumor size with time also.) To date most of the work has been done with a single high dose being administered to the test animals.

2. Divided Dose. Information is needed on the tumor yield when an animal is treated with a specific dose. That dose may be given as a single injection, or given over a period of time in smaller increments. These studies should include the effect of the time interval on the tumor yield; if a sparingly soluble compound or a pure metal is administered, the previous dose will not be excreted before the next injection is given.

3. Low Dose. It would be most useful to obtain data on the effect of giving animals a dose of a compound which would result in a tumor yield of 90+%, and then repeat these experiments with doses which are decreased by logarithmic units.

4. Routes Compared. It will be of interest to see what information is obtained if the same laboratory uses at least two routes to administer the same compound at equal dose levels. How does intramuscular compare with the oral route? Much more work must be done to evaluate the oral route. Here also, dose response studies must be made. With the exception of the chromates, the intratracheal route has been neglected. This route can give quite a bit of information about lung reactions, as well as the potential carcinogenicity of the compounds.

5. Compounds Compared. It is still unknown what conclusions can be drawn relative to a pure element if one of its compounds is found active.

6. Species Compared. To extrapolate information derived from animal studies to humans, much more data must be obtained on species to species variation. It is essential to know if the carcinogenic response of a rat or mouse is not the result of species specificity. Reviews are just now appearing on this subject (*83*).

7. Rates of Excretion. How rapidly are the metals excreted, and by what routes are they eliminated when pure metals are administered by gastrointestinal or by the parenteral routes?

8. Rates of Dissolution. How fast are compounds dissolved in the animal when given by the different routes? What is the chemical nature of the transported ion?

9. Artifacts. What artifacts are related to the experimental techniques. Are there similar effects to the "Oppenheimer Effect" yet to be observed? (As mentioned, the author believes the appearance of osteosarcomas may be an artifact.)

10. More Testing. What other industrial compounds are potential carcinogens?

11. *In vivo* Mechanisms. Can *in vivo* studies help determine the mechanism of action of this class of compounds, or must the work be done only in *in vitro* systems? Does a metal combination with a DNA in the test tube describe what goes on in the cell of a live treated animal?

REFERENCES

1. Pitot, H. C. Fundamentals of Oncology. Marcel Dekker, New York, 1978, pp. 32-33.
2. Sunderman, F. W., Jr. A review of the carcinogenicities of nickel, chromium and arsenic compounds in man and animals. Prev. Med. 5: 279 (1976).
3. Sunderman, F. W., Jr. Metal carcinogenesis in experimental animals. Food Cosmet. Toxicol. 9: 105 (1971).
4. Sunderman, F. W., Jr. Metal carcinogenesis. In: Advances in Modern Toxicology, Vol. 2. R. A. Goyer and M. A. Mehlman, Eds., John Wiley & Sons, New York, 1977.
5. Sunderman, F. W., Jr. Carcinogenic effect of metals. Fed. Proc. 37: 40 (1978).
6. Furst, A. The Chemistry of Chelation in Cancer. C. C. Thomas, Springfield, Ill., 1963, pp. 17-18.
7. Furst, A., and Haro, R. T. A survey of metal carcinogenesis. Progr. Exptl. Tumor Res. 12: 102 (1969).
8. Furst, A. Inorganic agents as carcinogens. In: Advances in Modern Toxicology, Vol. 3. M. A. Kraybill and M. A. Mehlman, Eds., Hemisphere Publishing Co., Washington, D.C., 1977, pp. 209-229.
9. Furst, A. An overview of metal carcinogenesis. In: Inorganic and Nutritional Aspects of Cancer. G. N. Schrauzer, Ed., Plenum Press, New York, 1978, pp. 1-12.
10. Furst, A., and Radding, S. B. Unusual metals as carcinogens. Biol. Trace Elem. Res. 1: 169 (1979).
11. Radding, S. B., and Furst, A. A review of metals and carcinogenicity. In: Molecular Basis of Environmental Toxicity, R. S. Bhatnagar, Ed., Ann Arbor Science Publishing, Ann Arbor, Mich., 1980, pp. 359-372.
12. International Agency for Research on Cancer. Some Inorganic and Organometallic Compounds. (IARC Monograph, Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 2), IARC, Lyon, 1973.
13. International Agency for Research on Cancer. Nickel and Nickel Compounds (IARC Monograph, Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 11), IARC, Lyon, 1976.
14. International Agency for Research on Cancer. Some Metals and Metallic Compounds. (Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 23). IARC, Lyon, 1980.
15. Furst, A., and Radding, S. B. An update on nickel carcinogenesis. In: Biogeochemistry of Nickel, J. A.

Niragn, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1980, pp., 585-600.
16. Payne, W. W. Carcinogenicity of nickel compounds in experimental animals. Proc. Am. Assoc. Cancer Res. 5: 50 (1964).
17. Furst, A. Tumorigenic effect of an organomanganese compound on F344 rats and Swiss albino mice. J. Natl. Cancer Inst. 60: 1171 (1978).
18. O'Gara, R. W., and Brown, J. M. Comparison of the carcinogenic actions of subcutaneous implants of iron and aluminum in rodents. J. Natl. Cancer Inst. 38: 947 (1967).
19. Nothdurft, H. Experimental production of sarcomas in rats and mice by implantation of round discs of gold, platinum, silver or ivory. Naturwiss. 42: 75 (1955).
20. Oppenheimer, B. S., Oppenheimer, E. T., Danishefsky, I., and Stout, A. P. Carcinogenic effect of metals in rodents. Cancer Res. 16: 439 (1956).
21. Nothdurft, H. Unterschiedliche Ausbeuten an subcutanen Fremdkorpersarcomen der Ratte in Abhangigkeit von Korperegion. Naturwiss. 49: 18 (1962).
22. Bryson, G., and Bischoff, F. Silicate-induced neoplasms. Progr. Exptl. Tumor Res. 11: 100 (1969).
23. Brand, K. G. Solid state or foreign body carcinogenesis. In: Scientific Foundations of Oncology, T. Symington and R. L. Carter, Eds., Wm. Heinemann Med. Book Ltd., London, 1976, pp. 490-495.
24. Furst, A., and Harding-Barlow, I. Interaction of metals and tissues. In: Advances in Modern Toxicology, Vol. I, New Concepts in Safety Evaluation, Part 2, M. A. Mehlman, R. E. Shapiro, and H. Blumenthal, Eds., Hemisphere, Washington, D.C., 1979, pp. 97-118.
25. Furst, A., Schlauder, M., and Sasmore, D. P. Tumorigenic activity of lead chromate. Cancer Res. 36: 1779 (1976).
26. Levy, L. S., and Clack, J. Further studies on the effect of cadmium on the prostate gland. I. Absence of prostatic changes in rats given oral cadmium sulphate for two years. Ann. Occup. Hyg. 17: 205 (1945).
27. Ambrose, A. W., Larson, P. S., Borzelleca, J. F., and Hennigar, G. R. Long term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13: 181 (1976).
28. Preussmann, R., and Ivankovic, S. Absence of carcinogenic activity in BD rats after oral administration of high doses of bismuth oxychloride. Food Cosmet. Toxicol. 13: 543 (1975).
29. Kroes, R., van Logten, M. J., Berkvens, J. M., DeVries, T., and van Esch, G. J. Carcinogenicity of lead arsenate and sodium arsenate and the possible synergistic effect of diethylnitrosamine. Food Cosmet. Toxicol. 12: 671 (1974).
30. Roe, F. J. C., Boyland, E., and Millican, K. Effects of oral administration of two tin compounds to rats over prolonged periods. Food Cosmet. Toxicol. 3: 277 (1965).
31. Boyland, E., Dukes, C. E., Grover, P. L., and Mitchley, B. C. V. The induction of renal tumors by feeding lead acetate to rats. Brit. J. Cancer 16: 283 (1962).
32. van Esch, G. J., van Gendren, H., and Vink, H. H. The induction of renal tumors by feeding basic lead acetate to rats. Brit. J. Cancer 16: 289 (1962).
33. van Esch, G. J., and Kroes, R. The induction of renal tumors by feeding basic lead acetate to mice and hamsters. Brit. J. Cancer 23: 765 (1969).
34. Schroeder, H. A., and Mitchener, M. Scandium, chromium (VI), gallium, yttrium, rhodium, palladium, indium in mice: effects on growth and life span. J. Nutr. 101: 1431 (1971).
35. Schroeder, H. A., and Mitchener, M. Selenium and tellurium in rats: effect on growth, survival and tumors. J. Nutr. 101: 1531 (1971).
36. Schroeder, H. A., Balassa, J. J., and Vinton, W. H. Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. J. Nutr. 83: 239 (1964).
37. Schroeder, H. A., and Mitchener, M. Life-term effects of mercury, methylmercury, and nine other trace metals on mice. J. Nutr. 105: 52 (1975).
38. Schroeder, H. A. Recondite toxicity of trace elements. In: Essays in Toxicology, Vol. 4, W. J. Hayes, Jr., Ed., Academic Press, New York, 1973, pp. 107-199.
39. Raabe, O. G. The generation of aerosols of fine particles. In: Fine Particles: Aerosol Generation, Measurement, Sampling and Analysis. B. Y. H. Liu, Ed., Academic Press, New York, 1976, pp. 57-110.
40. Brain, J. D., and Valberg, P. A. State of the art—deposition of aerosol in the respiratory tract. Am. Rev. Respir. Dis. 120: 1325 (1979).
41. Hueper, W. C. Experimental studies in metal carcinogenesis. IX. Pulmonary lesions in guinea pigs and rats exposed to prolonged inhalation of powdered metallic nickel. Arch. Pathol. 65: 600 (1958).
42. Saknyn, A. V., and Blokhin, V. A. Development of malignant tumors in rats exposed to nickel-containing aerosols. Vopr. Onkol. 24(4): 44 (1978).
43. Wehner, A. P., Busch, R. H., Olson, R. J., and Craig, D. K. Chronic inhalation of nickel oxide and cigaret smoke by hamsters. J. Am. Ind. Hyg. Assoc. 36: 801 (1975).
44. Ottolenghi, A. D., Haseman, J. K., Payne, W. W., Falk, H. L., and Macfarland, H. N. Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. J. Natl. Cancer Inst. 54: 1165 (1975).
45. Kovalenko, V. R., Isabekova, R. I., and Nemenko, B. A. Toxic and blastomogenic action of some chromium compounds. Zdravookhr. Kaz.(7): 50 (1972).
46. Wagner, W. D., Groth, D. H., Holtz, J. L., Madden, G. E., and Stockinger, H. E. Comparative chronic inhalation toxicity of beryl, with production of pulmonary tumors by beryl. Toxicol. Appl. Pharmacol. 15: 10 (1969).
47. Sanders, C. L., Cannon, W. C., and Powers, G. J. Lung carcinogenesis induced by inhaled high-fired oxides of beryllium and plutonium. Health Phys. 35: 193 (1978).
48. Laskin, S., Kuschner, M., and Drew, R. T. Studies in pulmonary carcinogenesis. In: Inhalation Carcinogenesis, P. Nettesheim, Ed., AEC Symposium Ser., Vol. 18, U.S. AEC, Oak Ridge, Tenn., 1970, pp. 321-350.
49. Furst, A., Cassetta, D. M., and Sasmore, D. P. Rapid induction of pleural mesotheliomas in the rat. Proc. West Pharmacol. Soc. 16: 150 (1973).
50. Bischoff, F., and Bryson, G. Toxicologic studies of tin needles at the intrathoracic site of mice. Res. Commun. Chem. Pathol. Pharmacol. 15: 331 (1976).
51. Hatch, T. F., and Gross, P. Pulmonary Deposition and Retention of Inhaled Aerosols. Academic Press, New York, 1964.
52. Saffiotti, U., Montesaro, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G. Respiratory tract carcinogenesis in hamsters induced by different numbers of administration of benzo(a)pyrene and ferric oxide. Cancer Res. 32: 1073 (1972).
53. Ho, W., and Furst, A. Intratracheal instillation method for mouse lungs. Oncology 27: 385 (1973).
54. Kasprzak, K. S., Marchow, L., and Breborowicz, J. Pathological reactions in rat lungs following intratracheal injection of nickel subsulfide and 3,4-benzpyrene. Res. Commun. Chem. Pathol. Pharmacol. 6: 237 (1973).
55. Lane, B. F., and Mass, M. J. Carcinogenicity and cocarcinogenicity of chromium carbonyl in heterotopic tracheal grafts. Cancer Res. 37: 1476 (1977).
56. Tsutomu, Y., and Nettesheim, P. Carcinogenicity of nickel subsulfide for respiratory tract mucosa. Cancer Res. 38:

3140 (1978).
57. Khachatrian, E. A. Study of the carcinogenic properties of chromium compounds by means of their action on mouse skin. Zh. Eksp. Klin. Med. 5: 88 (1965).
58. Gardner, L. U., and Heslington, H. F. Osteosarcoma from intravenous beryllium compounds in rabbits. Fed. Proc. 5: 221 (1946).
59. Schmähl, D., and Steinhoff, D. Experimental carcinogenesis in rats with colloidal silver and gold solutions. Z. Krebsforsch. 63: 586 (1960).
60. Lau, T. J., Hackett, R. L., and Sunderman, F. W., Jr. The carcinogenicity of intravenous nickel carbonyl in rats. Cancer Res. 32: 2253 (1972).
61. Druckrey, H., Hamperl, H., and Schmähl, D. Carcinogenic action of metallic mercury after intraperitoneal administration to rats. Z. Krebsforsch. 61: 511 (1957).
62. Rudali, G., and Silberman, C. L. Spontaneous carcinogenesis in mice following the administration of gold thioglucose. Rev. Fr. Etud. Clin. Biol. 11: 828 (1966).
63. Stoner, G. D., Shimkin, M. B., Troxell, M. C., Thompson, T. L., and Terry, L. S. Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. Cancer Res. 36: 1744 (1976).
64. Maltoni, C., Gualano, L., and Lefemine, G. Subcutaneous sarcomas oin rats following implantation of vitallium in different forms. In: Proceedings, 5th International Symposium on Biological Characterization of Human Tumours. Vol. I. Characterization of Human Tumours, W. Davis and C. Maltoni, Eds., American Elsevier, New York, 1974, pp. 123-124.
65. Ball, R. A., Van Gelder, G., Green, J. W., and Reece, W. O. Neoplastic sequelae following subcutaneous implantation of mice with rare earth metals. Proc. Soc. Exptl. Biol. Med. 135: 426 (1970).
66. Shabaan, A. A., Marks, U., Lancaster, M. W., and DuFeu, G. N. Fibrosarcoma induced by cobalt chloride ($CoCl_2$) in rats. Lab. Anim. 11: 43 (1977).
67. Gunn, S. A., Gould, T. C., and Anderson, W. A. D. Effect of zinc on carcinogenesis by cadmium. Proc. Soc. Exptl. Biol. 115: 653 (1964).
68. Gilman, W. Metal carcinogenesis. II. Study on the carcinogenicity of cobalt, copper, iron and nickel compounds. Cancer Res. 22: 158 (1962).
69. Furst, A., and Schlauder, M. D. Inactivity of two noble metals as carcinogens. J. Environ. Pathol. Toxicol. 1: 51 (1977).
70. Kazantzis, G., and Hanbury, W. J. The induction of sarcoma in the rat by cadmium sulphide and by cadmium oxide. Brit. J. Cancer 20: 190 (1966).
71. Hueper, W. C. Experimental studies in metal carcinogenesis. IV. Cancer produced by parenterally induced metallic nickel. J. Natl. Cancer Inst. 16: 55 (1955).
72. Mazabraud, A. Experimental production of bone sarcomas in the rabbit by a single local injection of beryllium. Bull. Cancer (Paris) 62: 49 (1975).
73. Michalowski, I. Die experimentelle Erzeugung einer teratoiden Neubildung der Hoden beim Hahn. Zentralbl. Allg. Pathol. Pathol. Anat. 38: 585 (1926).
74. Falin, L. I., and Gromtseva, K. E. The pathogenesis of experimental teratoid tumors of the genital glands. I. Experimental zinc sulfate teratoma of the testicle of roosters. Arch. Sci. Biol. USSR 56: 101 (1939).
75. Falin, L. I., and Annissimova, V. V. Pathogenese der experimentellen teratoiden Geschwüllste der Geshlechtsdrüsen. Teratoide Hodengechwulst beim Hahn, Erzeugt durch Einfuhrung von $CuSO_4$-Losung. Z. Krebsforsch. 50: 339 (1940).
76. Riviere, M. R., Chouroulinkov, I., and Guerin, M. Testicular tumors in the rat after injection of zinc chloride. Compt. Rend. 240: 2649 (1959).
77. Damajanov, K., Sunderman, F. W., Jr., Mitchell, J. M., and Allpass, P. R. Induction of testicular sarcomas in Fischer rats by intratesticular injection of nickel subsulfide. Cancer Res. 38: 268 (1978).
78. Jasmin, G., and Riopelle, J. L. Renal carcinomas and erythrocytosis in rats following intrarenal injection of nickel subsulfide. Lab. Invest. 35: 71 (1976).
79. Sosinski, E. Morphological changes in rat brain and skeletal muscle in the region of nickel oxide implantation. Neuropatol. Pol. 13: 479 (1975).
80. Rencher, A. C., Carter, M. W., and McKee, D. W. A retrospective epidemiological study of mortality at a large western copper smelter. J. Occup. Med. 19: 754 (1977).
81. Ohsaki, Y., Abe, S., Kimura, K., Tsuneta, Y., Mikami, H., and Murao, M. Lung cancer in Japanese chromate workers. Thorax 33: 372 (1978).
82. Berg, J. W., and Burbank, F. Correlations between carcinogenic trace metals in water supplies and cancer mortality. Ann. N.Y. Acad. Sci. 199: 249 (1972).
83. Crouch, E., and Wilson, R. Interspecies comparison of carcinogenic potency. J. Toxicol. Environ. Health 5: 1095 (1979).