

Experimental Identification of Chemical Carcinogens, Risk Evaluation, and Animal-to-Human Correlations

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Experimental methods for the identification of chemical carcinogens have been extensively developed, including animal bioassay methods, animal models for cancer induction at major organ sites, models for the study of the effects of carcinogens in cells and tissues in culture and methods for the study of molecular events (metabolic activation, binding and detoxification of carcinogens; DNA damage and repair; mutagenicity). Current sources of documentation on carcinogenicity data are reviewed. The number of "known carcinogens" will vary considerably, depending on the criteria adopted for accepting evidence of carcinogenicity.

Criteria for the evaluation of risks, benefits, and technological alternatives for public policy on environmental carcinogens are reviewed and the following steps discussed: registration of environmental chemical carcinogens and their uses; risk evaluation (considering sources, adequacy, quality and limits of the evidence; quantitative dose-response extrapolation within the same biological system; and species and model conversion factors); benefits evaluation; analysis of technological alternatives; comparative judgment and decision; open public documentation.

The problem of animal-to-human correlations is considered, particularly for respiratory carcinogenesis. A laboratory approach is reviewed which includes: development and study of whole animal models for carcinogenesis, analysis of animal tissue responses to carcinogens *in vivo* and through *in vitro* culture methods for morphological and biochemical studies, and development of *in vitro* culture methods for human target tissues. This approach is aimed at providing an experimentally controlled and quantifiable method for the correlation of animal and human observations in carcinogenesis.

Identification of Chemical Carcinogens: Experimental Methods

Methods for identifying the carcinogenicity of chemicals are based on different levels of observation: human epidemiology and pathology, whole animal studies, *in vitro* cellular studies, and mutagenesis and molecular interaction studies. Human and/or whole animal evidence, when adequately substantiated, is the presently established basis for classifying a chemical as a carcinogen. Evidence based on cellular transformation, mutagenesis, chemical structure and chemical reac-

tivity is now providing suggestive or supportive data, but the predictiveness of these methods is still in the process of being validated (1, 2).

Epidemiologic evidence, even with its limitations, has pointed to clear trends which indicate the major role of environmental agents in the causation of human cancer (3-5).

Experimentation in animal models allowed us to observe a close similarity between the development of cancers in the laboratory animals and in their human counterpart. The development of animal models for carcinogenesis represents a major methodological step in the investigation of the effects of carcinogens on their target tissues, as well as a powerful tool in defining their metabolic pathways and the permissive host conditions required for a carcinogenic response (2).

Most of the major types of human cancers have been reproduced in animal models by chemical in-

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duction. Their pathologic characteristics closely resemble those known from human pathology. This close correlation by itself strongly supports the evidence for a chemical origin of a large proportion of human cancers (6).

Great progress was made in the last decade in the development of models for the study of neoplastic transformation induced by chemical and physical agents in cells in culture. These methods are now undergoing evaluation to determine their reproducibility and reliability for use both in carcinogenesis research and for bioassays. The development, definition and validation of short-term screening methods for carcinogenesis were recently reviewed at a conference organized by the IARC in Brussels in June, 1975 (7), and at a Seminar and Workshop held at the Given Institute of Pathobiology in Aspen, Colorado, in July, 1976 (8).

Molecular events in carcinogenesis have also been studied, including those that lead to a critical DNA damage and can be expressed as mutations and those affecting the metabolic activation and detoxification of carcinogens, their interaction and binding with target macromolecules, the damage and repair of DNA as well as the effects on cell regulatory control mechanisms.

There is a great need now to correlate the effect of carcinogens through all levels of observation, from the human and the whole animal level to the tissue and cellular level and to the molecular level.

For the purpose of defining chemicals as carcinogens, it is important to consider both the quality of the evidence and its extent (2).

Bioassay procedures, initially outlined by several expert committees (9-14) were subsequently further developed and specified in much greater detail by the NCI Carcinogenesis Program as guidelines for carcinogen bioassays (15). A model for the fully detailed publication of the reports of such bioassays was also established (16).

Documentation of chemical carcinogenicity data is provided by the following sources: (1) IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, published by the International Agency for Research on Cancer (17) which are, at present, the only extensive reference source of carcinogenicity data evaluated by a systematic process of critical review of the experimental conditions, bioassay protocols, standards of pathology and results; (2) Survey of Compounds Which Have Been Tested for Carcinogenic Activity," published by the National Cancer Institute (18) which constitutes a key documentation source, but without critical evaluation of the literature data; (3) Suspected Carcinogens, a Subfile of the NIOSH Toxic Substance List, published by the National Institute for

Occupational Safety and Health (19) which lists about 1500 chemicals reported in the literature as having "neoplastic" or "carcinogenic" effects, with no critical evaluation; (4) other lists of carcinogens compiled by various individual authors or groups in several books on carcinogenesis.

I believe that it is very important that all these sources be used with great caution, particularly when they have not been assembled through a critical review process.

The number of "known carcinogens" will vary considerably, depending on the degree of critical stringency adopted for accepting evidence of carcinogenicity (2).

Criteria were recently proposed (6) for the classification of the evidence of carcinogenicity into the following three categories: positive, negative under the conditions of observation, and inconclusive.

Evaluation of Risks, Benefits and Technological Alternatives for Public Policies on Environmental Carcinogenesis

In the implementation of public health policies, a society has to make operational choices and decisions. Implicit in them is a judgment on the balance of benefits and risks; in addition, I believe that particular attention should be given to a choice of technological alternatives. These evaluations are difficult and usually based on "soft" evidence: errors in judgment can lead to major human suffering and loss. If we accept, as a society, that cancer prevention is both feasible and needed, then we should commit ourselves to the side of prudence in our judgment and make sure that we avoid underestimating the risk and that we avoid overestimating the benefits. A special policy decision is required when the evidence for the carcinogenicity of an agent is inconclusive. The most "prudent" policy is to consider all agents, for which the evidence is not clearly negative under accepted minimum conditions of observation, as if they were positive: this policy may not always be applicable under practical conditions, but when applicable it will ensure maximum protection. In other words, for a prudent toxicological policy a chemical should be considered guilty until proven innocent (20). The least prudent policy is to consider all chemicals which have not been conclusively proven positive as if they were negative, including those for which various levels of suspicion may exist and may eventually be confirmed by subsequent more adequate observations. These opposite attitudes are reflected in the

approaches to legislation and regulation of carcinogens in different countries and for different types of exposure.

The analysis of risks and the analysis of benefits are two separate endeavors, based on different principles, using different methods and concerning different disciplines. A lot of confusion and controversies have arisen from the interferences of one of these evaluations into the domain of the other. It is essential that the integrity and objectivity of these analyses be ensured and that no pressure be exerted on their scientific independence and impartiality.

The evaluation of the balance of benefits and risks for public policy requires, in my view, the following necessary and separate steps.

Registration of Environmental Chemical Carcinogens and Their Uses

We know very little, as individuals, of what hits us in our everyday life. The available information basis is still scanty. The Carcinogenesis Program of the National Cancer Institute, through support of a collaborative research project at the Stanford Research Institute, has endeavored to obtain data on the environmental distribution and human exposure levels of large numbers of environmental chemicals, and has contributed this information to the IARC Monographs (17). In the United States, the Toxic Substances Control Act of 1976 contains a provision for notification to the Government of new chemicals and new major uses of chemicals. This will provide a much needed basis of knowledge on the nature and extent of chemical exposures in our environment. More specifically, in order to assess the population risk from chemical carcinogens, I believe that it would be extremely useful to establish a registration of all uses of products that are known to be carcinogenic.

Risk Evaluation

The methods for evaluating cancer risks in human populations from known or potential exposures to carcinogens are still poorly defined and mostly provide soft evidence. It is therefore essential that highly qualified professional expertise be used to define the extent of arbitrariness and uncertainty inherent in the process. A constant guideline should be not to underestimate the risk. Critical issues in risk assessment methodology have been recently reviewed (21-24). The following aspects need to be considered.

(1) Sources of evidence should include extent and definition of exposure in humans, epidemiologic evidence of risk, and experimental evidence of risk.

(2) Analysis of the adequacy and quality of the evidence should provide a critical assessment of methodologies including detailed reviews of bioassay protocols and results of the extent of documentation.

on the basis of the limited sensitivity of each of the methods used (e.g., sample size, extent of observations, extent of controls): this consideration is particularly important in evaluating apparently negative results which are always limited by the sensitivity of the method and therefore by the detectability of the effect.

(4) Quantitative dose-response extrapolations should be confined to extrapolations from known exposure with known responses to much lower levels of exposure with unknown responses, within the same biological system. Several mathematical models have been proposed for this extrapolation and it is important that they be chosen so that they would not underestimate the projected risk.

(5) Species conversion factors should be included in estimating risk levels for one species (e.g., humans) from data obtained in another species. Many variables combine in determining such a species conversion factor, such as body surface, body weight, metabolic pathways, nutritional conditions, genetic variability, bacterial flora, tissue distribution, retention and fate of the chemical, etc. It is extremely difficult to arrive at precise numerical values in such a conversion procedure without precise comparative measurements of specific variables in the two species, including an estimate of their interindividual variability. No indication is presently available of the extent of sampling necessary for an adequate evaluation of a human population in this field. For exposures to the general population, one should consider all ages, transplacental exposures, concurrent disease conditions, and special susceptibility states. In the absence of precise objective data, one should clearly state the adopted limits of uncertainty and provide for a large margin of error in each determination, always on the side of not underestimating the risk.

(6) Model conversion factors should also be included to account for the role of additional variables when observations were obtained from exposure conditions in one species that are markedly different from those in the population (e.g., consider different routes or modes of exposures, vehicles, modifying factors, variations in age, sex, perinatal exposures, disease states, single versus multiple exposures, etc.) Hard data are rarely available and prudent judgment of these factors is required.

In conclusion, at the present time there does not appear to be any reliable method for obtaining a direct estimate of risk and the required confidence

limits, applicable to a population for a given exposure to a carcinogen, except by direct observations of exposed populations after the fact. Risk evaluation is of such an elusive nature that the best that science can do, at the present state of our knowledge, is to attempt to estimate an upper bound of risk, and even this with all the caveats mentioned above. I believe that it is essential that all the steps that have gone into the final estimate of risk, or of its upper bound, be documented and defined in each case, so that they can be corrected when new information becomes available.

Benefits Evaluation

A critical analysis of the benefits that may derive from the production and use of an agent needs to be made by experts in all the relevant fields in each case. Benefits can be of a direct or indirect nature and they can affect the technology, the economy and the health of populations and of selected individuals. They need to be precisely defined and documented for each of these aspects by appropriate and rigorous methods. The adequacy, quality, and reliability of this documentation must be assessed. In the evaluation of benefits it is important to identify the individuals or groups in a population which will receive the projected benefits and to determine whether they are the same that will be also affected by the risks.

Analysis of Technological Alternatives

In the evaluation of the balance of benefits and risks, critical consideration must be given to all the options presented by technological alternatives, ranging from adoption of different technologies, to product replacement and to effective reduction or elimination of specific exposures. Replacement of a chemical having a certain estimated carcinogenic risk with another chemical of unknown risk may mean the adoption of a more hazardous alternative; therefore the adoption of alternate products or conditions must be carefully evaluated for its total impact on health as well as other societal needs. For each technological alternative under consideration, documentation of all the aspects of risk and benefits evaluations should be obtained and critically reviewed.

The estimated least hazardous technological alternative should be identified and the feasibility of its implementation should be determined, in order to select the best technology acceptable for implementation by society.

Comparative Judgment and Decision

When objective critical reviews and assessments have been obtained, separately, on benefits, risks and technological alternatives, then comparative judgments and operational decisions have to be made with a sound understanding of the factors involved and with a far-reaching view of the societal implications of these decisions in terms of public policy. This process is really a policy choice.

Consideration should be given to the voluntary or involuntary nature of the individual exposures and to the intentional or unintentional nature of human exposures resulting from the production or use of a given chemical. The mechanisms by which society will implement and accept a selected technology will have to be identified (e.g., legislation, regulation, legal action, education, voluntary action, advertising, etc.). The responsibilities of society to the individuals placed at higher risk, particularly if they are not the recipients of benefits, have to be considered.

Open Public Documentation

Many selected technologies may retain an inherent level of risk, which should be openly stated. The entire documentation used in this process should be made public. The extent of arbitrariness and uncertainty in this documentation should be clearly stated and attention should be given to identifying gaps in our knowledge which, if filled, could provide a new basis for decision. Each case should be reviewed periodically.

In conclusion, having determined social acceptability, the selected technological alternative should be implemented, the public fully informed, the implementation monitored and its consequences observed. The whole problem of evaluation of environmental hazards is at the interface of science and policy. The objectivity of scientific observation should be rigorously preserved. Recognition of the etiological role of an environmental agent for a disease condition elicits an ethical problem. The relationships of etiology and ethics were recently discussed (24).

Animal-to-Human Correlations, with Special Emphasis on Respiratory Carcinogenesis

We do not presently have any reliable method for a direct quantitative extrapolation from animal experiments to human carcinogenic hazards. Mathematical approaches alone are inadequate to

cope with the problem, but they suggest that any amount of carcinogen, however small, will contribute to the total carcinogenic effect in the population by raising, however slightly, the level of response of the total population (6, 21, 23).

The problem of animal-to-human correlations, in my view, is centered on bridging the gap between whole-animal experiments and human pathology by devising a step-by-step analysis of the biological response under gradually different but closely comparable conditions. These comparative studies begin with the development of whole-animal models, continue with studies on the response of specific animal tissues, then extend to *in vitro* systems for the same animal tissues and cells in culture, and finally reach the study of the corresponding human tissues and cells in culture and their correlation to human pathology *in vivo*.

Our laboratories have developed this approach, with particular emphasis on respiratory tract carcinogenesis studies. The sequence of research methods developed in this area is now being extended also to other tissues and organs (2, 6, 25).

We first developed methods for the induction of respiratory tract cancers in animals, including bronchogenic, tracheal, and laryngeal carcinomas, closely resembling their human counterpart. Their induction was obtained by exposure to polycyclic hydrocarbon carcinogens carried by particulate materials. Morphological characterization, dose-response relationships, the role of particulate carriers and susceptibility factors were defined in this model (26-34).

This model has been largely used in a series of subsequent studies by several investigators. Similar methods were developed in other laboratories (35-37). Systemically acting respiratory carcinogens were also identified (38-41). Synergistic effects between topical and systemic carcinogens were observed (30, 40, 42). Histogenesis studies, at the optical and electron-microscopic level, defined the early stages of tissue response (43-48).

Methods were then developed for the analysis of cellular structures and of morphological-biochemical correlations in the response of target epithelia to carcinogens *in vivo* or in organ culture (49-53). Cellular control mechanisms, capable of inhibiting the carcinogenic effect, were identified and studied; this approach, both at the cellular and at the whole animal levels, brought on the discovery of the anticarcinogenic activity of vitamin A and derivatives (retinoids) including a number of synthetic products of high biological activity (27, 45, 51, 54-58).

Finally in the last few years, the development of *in vitro* culture methods for human tissues has made it possible to extend the focus of these studies to the

direct observation of the effects of carcinogens in human target tissues, under controlled experimental conditions in organ and cell culture.

Human bronchial epithelium, maintained in culture for as long as six months, has been used in a variety of short-term and long-term studies in our laboratory (59-63). From this work a pattern of response of the human target tissue to respiratory carcinogens is emerging, showing a high degree of individual variability in the level of carcinogen bound to DNA in human bronchial epithelia but showing a basic similarity of the pathways of metabolic activation of carcinogens between animal and human tissues. The specific principal adduct formed in the binding of benzo[a]pyrene to DNA has been identified in animal and human bronchial cells (64).

The characteristics of individual susceptibility and the effects of different carcinogens directly on human respiratory tissues are thus in the process of being identified and quantitatively defined.

The development of these new research methods is expected to provide an entirely new, experimentally controlled and quantifiable approach to the old problem of how to correlate animal and human observations in carcinogenesis (2).

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REFERENCES

1. Saffiotti, U. Validation of short-term bioassays as predictive screens for chemical carcinogens. In: Screening Tests in Chemical Carcinogenesis. R. Montesano, H. Bartsch and L. Tomatis, Eds. IARC Scientific Publications No. 12, International Agency for Research on Cancer, Lyon, France, 1976.
2. Saffiotti, U. *In vitro* carcinogenesis methods in relation to the development of carcinogenesis research. In: *In Vitro Carcinogenesis. Guide to the Literature, Recent Advances and Laboratory Procedures*. U. Saffiotti and H. Autrup, Eds., No. 44, DHEW Publ. No. (NIH) 78-844, Washington, D. C., 1978, p. 1.
3. Fraumeni, J. F., Jr., ed. *Persons at High Risk of Cancer. An Approach to Cancer Etiology and Control*. Academic Press, New York, 1975.
4. Levin, D. L., et al. *Cancer Rates and Risks*. 2nd Ed., U. S. Dept. of Health Education and Welfare, DHEW Publ. No. (NIH) 75-691, Washington, D. C., 1974.
5. Saffiotti, U., and Wagoner, J. K., Eds. *Occupational Carcinogenesis*. Ann. N. Y. Acad. Sci. 271: (1976).
6. Saffiotti, U. Identifying and defining chemical carcinogens. In: *Origins of Human Cancer*. H. Hiatt, J. D. Watson, and J. Winsten, Eds. (Cold Spring Harbor Conferences on Cell Proliferation, Vol. 4), Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y., 1977.
7. Montesano, R., Bartsch, H., and Tomatis, L., Eds. *Screening Tests in Chemical Carcinogenesis*. IARC Scientific Publ. No. 12, International Agency for Research on Cancer, Lyon, France, 1976.

8. Saffiotti, U., and Autrup, H., Eds. *In Vitro* Carcinogenesis. Guide to the Literature, Recent Advances and Laboratory Procedures. NCI Carcinogenesis Tech. Rept. Ser. No. 44, DHEW Publ. No. (NIH) 78-844, Washington, D. C., 1978.
9. International Union Against Cancer. Report of symposium on potential cancer hazards from chemical additives and contaminants to foodstuffs. *Acta Unio Intl. Contra Carcum* 13: 170 (1957).
10. Food Protection Committee, Food and Nutrition Board. Problems in the evaluation of carcinogenic hazards from the use of food additives. National Academy of Sciences—National Research Council Publ. No. 749 (1960); *Cancer Res.* 21: 429 (1961).
11. Fifth Report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Carcinogenic Hazards of Food Additives. WHO Tech. Rept. Ser. 220, World Health Organization, Geneva (1961).
12. Berenblum, I., Ed., *Carcinogenicity Testing*. UICC Tech. Rept. Ser. Vol. 2, International Union Against Cancer, Geneva, 1969.
13. Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation. Panel on carcinogenesis report on cancer testing in the safety evaluation of food additives and pesticides. *Toxicol. Appl. Pharmacol.* 20: 419 (1971).
14. *The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity*. Canadian Government Department of Health and Welfare, Ottawa, Canada, 1973.
15. Sontag, J. M., Page, N. P., and Saffiotti, U. Guidelines for carcinogen bioassay in small rodents. NCI Carcinogenesis Tech. Rept. Ser. No. 1, DHEW Publication No. (NIH) 76-801, U. S. Government Printing Office, Washington, D. C., 1976.
16. National Cancer Institute. Carcinogenesis bioassay of trichloroethylene. NCI Carcinogenesis Tech. Rept. Series, No. 2. DHEW Publ. No. (NIH) 76-802 Washington, D. C., 1976.
17. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risk of chemicals to man. Lyon, France, Vol. 1 (1972), Vol. 2 (1973), Some Inorganic and Organometallic Compounds; Vol. 3 (1973), Some Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds; Vol. 4 (1974), Some Aromatic Amines, Hydrazine and Related Substances, *N*-Nitroso Compounds and Miscellaneous Alkylating Agents; Vol. 5 (1974), Some Organochlorine Pesticides; Vol. 6 (1974), Sex Hormones; Vol. 7 (1974), Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals; Vol. 8 (1975), Some Aromatic Azo Dyes; Vol. 9 (1975), Some Aziridines, *N*-, *S*- and *O*-Mustards and Selenium; Vol. 10 (1976), Some Naturally Occurring Substances; Vol. 11 (1976), Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anesthetics; Vol. 12 (1976), Some Carbamates, Thiocarbamates and Carbazides.
18. U. S. Department of Health, Education, and Welfare, National Institutes of Health, National Cancer Institute. Survey of Compounds Which Have Been Tested for Carcinogenic Activity (Public Health Service Publ. No. 149), U. S. Government Printing Office, Washington, D. C. Hartwell, J. L., Original (1951); P. Shubik and J. L. Hartwell, Suppl. 1 (1957); P. Shubik, J. L. Hartwell and J. A. Peters, Eds., Suppl. 2 (1969); 1961-1967 Vol. (1973); 1968-1969 Vol. (1971); 1970-1971 Vol. (1974); 1971-1973 Vol. (1976).
19. National Institute for Occupational Safety and Health, Center for Disease Control, U. S. Department of Health, Education, and Welfare. Suspected Carcinogens. A Subfile of the NIOSH Toxic Substances Lists, DHEW Publ. No. (NIOSH) 75-188, Rockville, Md., 1975.
20. Ad Hoc Committee on the Evaluation of Low Levels of Environmental Carcinogens. Report to the Surgeon General, USPHS: Evaluation of environmental carcinogens. In: *Chemicals and the Future of Man*. Hearings before the Subcommittee on Executive Reorganization and Government Research of the Committee on Governmental Operations of the U. S. Senate, 92nd Congress, 1st Session: 171. U. S. Govt. Printing Office, Washington, D. C., 1971.
21. Hoel, D. G., et al. Estimation of risks of irreversible, delayed toxicity. *J. Toxicol. Environ. Health* 1: 133 (1975).
22. Saffiotti, U. Risk-benefit considerations in public policy on environmental carcinogenesis. In: *Proceedings of the Eleventh Canadian Cancer Research Conference*. National Cancer Institute of Canada, Toronto, Ontario, Canada, 1976, pp. 13-11.
23. Crump, K. S., et al. Fundamental carcinogenic processes and their implications for low dose risk assessment. *Cancer Res.* 36: 2973 (1976).
24. Saffiotti, U. Scientific bases of environmental carcinogenesis and cancer prevention: developing an interdisciplinary science and facing its ethical implications. *J. Toxicol. Environ. Health* 2: 1435 (1977).
25. Saffiotti, U. The laboratory approach to the identification of environmental carcinogens. In: *Proceedings of the Ninth Canadian Cancer Research Conference*. P. J. Scholefield, Ed., University of Toronto Press, Toronto, Canada, 1972, p. 23.
26. Saffiotti, U., et al. Experimental studies of the conditions of exposure to carcinogens for lung cancer induction. *J. Air Pollut. Control Assoc.* 15: 23 (1965).
27. Saffiotti, U., et al. Studies on experimental lung cancer: Inhibition by vitamin A of the induction of tracheobronchial squamous metaplasia and squamous cell tumors. *Cancer* 20: 857 (1967).
28. Saffiotti, U., Cefis, F., and Kolb, L. H. A method for the experimental induction of bronchogenic carcinoma. *Cancer Res.* 28: 104 (1968).
29. Saffiotti, U. Experimental respiratory tract carcinogenesis and its relation to inhalation exposures. In: *Inhalation Carcinogenesis*. M. G. Hanna, Jr., P. Nettesheim, and J. R. Gilbert, Eds., AEC Symposium Series, No. 18 (CONF-691001), U. S. Atomic Energy Commission Division of Technical Information Extension, Oak Ridge, Tenn., 1970, p. 27.
30. Montesano, R., Saffiotti, U., and Shubik, P. The role of topical and systemic factors in experimental respiratory carcinogenesis. In: *Inhalation Carcinogenesis*. M. G. Hanna, Jr., P. Nettesheim, and J. R. Gilbert, Eds., AEC Symp. Ser., No. 18 (CONF-691001), U. S. Atomic Energy Commission, Division of Technical Information Extension, Oak Ridge, Tenn., 1970, p. 353.
31. Saffiotti, U. Morphology of respiratory tumors induced in Syrian golden hamsters. In: *Morphology of Experimental Respiratory Carcinogenesis*. P. Nettesheim, M. G. Hanna, Jr., and J. W. Deatherage, Jr. AEC Symp. Ser. No. 21 (CONF-700501), U. S. Atomic Energy Commission, Division of Technical Information Extension, Oak Ridge, Tenn., 1970, p. 254.
32. Saffiotti, U., et al. Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[*a*]pyrene and ferric oxide. *Cancer Res.* 32: 1073 (1972).
33. Saffiotti, U., et al. Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[*a*]pyrene and ferric oxide. *J. Natl. Cancer Inst.* 49: 1199 (1972).
34. Saffiotti, U., and Kaufman, D. G. Carcinogenesis of laryngeal carcinoma. *Laryngoscope* 85: 454 (1975).
35. Hanna, M. G., Jr., Nettesheim, P., and Gilbert, J. R., Eds. *Inhalation Carcinogenesis*. AEC Symp. Ser. No. 18

- (CONF-691001), U. S. Atomic Energy Commission, Division of Technical Information Extension, Oak Ridge, Tenn., 1970.
36. Nettesheim, P., Hanna, M. G., Jr., and Deatherage, J. W., Jr., Eds. Morphology of Experimental Respiratory Carcinogenesis. AEC Symp. Ser., No. 21 (CONF-700501), U. S. Atomic Energy Commission, Division of Technical Information Extension, Oak Ridge, Tenn., 1970.
 37. Karbe, E., and Park, J. F., Eds. Experimental Lung Cancer. Carcinogenesis and Bioassays. Springer-Verlag, Berlin-Heidelberg-New York, 1974.
 38. Montesano, R., and Saffiotti, U. Carcinogenic response of the respiratory tract of Syrian golden hamsters to different doses of diethylnitrosamine. *Cancer Res.* 28: 2197 (1968).
 39. Montesano, R., and Saffiotti, U. Carcinogenic response of the hamster respiratory tract to single administration of diethylnitrosamine given at birth. *J. Natl. Cancer Inst.* 44: 413 (1970).
 40. Montesano, R., et al. Brief communication: synergistic effects of benzo[a]pyrene and diethylnitrosamine on respiratory carcinogenesis in hamsters. *J. Natl. Cancer Inst.* 53: 1395 (1974).
 41. Magee, P. N., Montesano, R., and Preussman, R. *N*-Nitroso compounds and related carcinogens. In: *Chemical Carcinogens*. C. E. Searle, Ed., ACS Monograph 173, American Chemical Society, Washington, D. C., 1976, p. 491.
 42. Kaufman, D. G., and Madison, R. M. Synergistic effects of benzo[a]pyrene and *N*-methyl-*N*-nitrosourea on respiratory carcinogenesis in Syrian golden hamsters. In: *Experimental Lung Cancer. Carcinogenesis and Bioassays*. E. Karbe and J. F. Park, Eds., Springer-Verlag, Berlin-Heidelberg-New York, 1974, p. 207.
 43. Saffiotti, U., Cefis, F., and Shubik, P. Histopathology and histogenesis of lung cancer induced in hamsters by carcinogens carried by dust particles. In: *Lung Tumors in Animals*. L. Severi, Ed. Division of Cancer Research, University of Perugia, Perugia, Italy, 1966, p. 537.
 44. Harris, C. C., et al. Acute ultrastructural effects of benzo[a]pyrene and ferric oxide on the hamster tracheobronchial epithelium. *Cancer Res.* 31: 1977 (1972).
 45. Harris, C. C., et al. Histogenesis of squamous metaplasia in the hamster tracheal epithelium caused by vitamin A deficiency or benzo[a]pyrene-ferric oxide. *J. Natl. Cancer Inst.* 48: 743 (1972).
 46. Harris, C. C., et al. Histogenesis of squamous metaplasia and squamous cell carcinoma of the respiratory epithelium in an animal model. *Cancer Chemother. Repts.* 4: 43 (1973).
 47. Harris, C. C., et al. Ultrastructural effects of *N*-methylnitrosourea on the tracheobronchial epithelium of the Syrian golden hamster. *Int. J. Cancer* 12: 259 (1973).
 48. Harris, C. C., et al. Atypical cilia in the tracheobronchial epithelium of the hamster during respiratory carcinogenesis. *J. Pathol.* 114: 17 (1974).
 49. Smith, J. M., et al. Isolation of enzymatically active nuclei from epithelial cells of the trachea. *Cancer Res.* 31: 199 (1971).
 50. Kaufman, D. G., et al. Coordinated biochemical and morphologic examination of hamster tracheal epithelium. *J. Natl. Cancer Inst.* 49: 783 (1972).
 51. Kaufman, D. G., et al. RNA metabolism in tracheal epithelium: alteration in hamsters deficient in vitamin A. *Science* 177: 1105 (1972).
 52. Kaufman, D. G., et al. Binding of ³H-labeled-benzo[a]pyrene to DNA in hamster tracheal epithelial cells. *Cancer Res.* 33: 2837 (1973).
 53. Harris, C. C., et al. Localization of benzo[a]pyrene-³H and alterations in nuclear chromatin caused by benzo[a]pyrene-ferric oxide in the hamster respiratory epithelium. *Cancer Res.* 33: 2842 (1973).
 54. Clamon, G., et al. α - and β -retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture. *Nature* 250: 64 (1974).
 55. Genta, V. M., et al. Vitamin A deficiency enhances binding of benzo[a]pyrene to tracheal epithelial DNA. *Nature* 247: 48 (1974).
 56. Sporn, M. B., et al. The reversal of keratinized squamous metaplastic lesions of vitamin A deficiency in tracheobronchial epithelium by vitamin A and vitamin A analogs in organ culture: a model system for anti-carcinogenesis studies. In: *Experimental Lung Cancer. Carcinogenesis and Bioassays*. E. Karbe and J. F. Park, Eds., Springer-Verlag, Berlin-Heidelberg-New York, 1974, p. 575.
 57. Sporn, M. B., et al. Activity of vitamin A analogues in cell cultures of mouse epidermis and organ cultures of hamster trachea. *Nature* 253: 47 (1975).
 58. Sporn, M. B., et al. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Fed. Proc.* 35: 1332 (1976).
 59. Harris, C., et al. Carcinogenic polynuclear hydrocarbons bind to macromolecules in cultured human bronchi. *Nature* 252: 68 (1974).
 60. Harris, C. C., et al. Binding of [³H]benzo[a]pyrene to DNA in cultured human bronchus. *Cancer Res.* 36: 1011 (1976).
 61. Harris, C. C., et al. Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi. *Science* 194: 1067 (1976).
 62. Harris, C. Chemical carcinogenesis and experimental models using human tissues. *Beitr. Pathol.* 158: 389 (1976).
 63. Harris, C. C., et al. Explant culture and xeno-transplantation of human bronchi. In: *In Vitro Carcinogenesis. Guide to the Literature, Recent Advances and Laboratory Procedures*. U. Saffiotti and H. Autrup, Eds., NCI Carcinogenesis Tech. Rept. Ser. No. 44, DHEW Publ. No. (NIH) 78-844, Washington, D. C., 1978, p. 144.
 64. Jeffrey, A. M., et al. Structures of benzo[a]pyrene-nucleic acid adducts formed in human and bovine bronchial explants. *Nature* 269: 348 (1977).