Application of Biological Markers to Carcinogen Testing

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Welcome to the Office of Toxic Substances' Conference on the "Application of Biological Markers to Carcinogen Testing." This meeting marks the signal development in the evolution of the application of science at the Office of Toxic Substances of the United States Environmental Protection Agency (U.S. EPA). The tone of the conference can perhaps best be expressed by the words of Henry Pitot in the second edition of his Fundamentals of Oncology, "Although the production of neoplasia in animals at a statistically higher level than in controls has been considered indicative of carcinogenicity of the agent under study, modern concepts of the natural history of neoplastic development require that this simplistic evaluation of the data be reconsidered." In other words, it is time that we look beyond the "black box" approach to carcinogen testing and evaluation.

Undoubtedly the rodent model will remain at the core of bioassay programs for the detection of chemicals which have carcinogenic potential, but we must begin to use intelligently the scientific tools that are available to understand and unravel the "how" and the "why" of tumorgenic responses. Along this line, Weisburger and Williams have proposed a five-subcategory scheme for the classification of carcinogens, based largely on putative mechanisms whereby these carcinogenic agents may act. Certainly at this time there is not sufficient evidence on the mechanisms of carcinogenic action of chemicals for one to construct and employ definitive categories such as these, but it helps to point out the strong suggestive evidence that there are, in fact, different types of carcinogens and different modalities of tumorgenic induction. If we fail to use the scientific tools available to us, we will remain in what Phillipe Shubick has very aptly termed "the pre-history of carcinogenesis."

We at EPA remain committed to a preventative stance with respect to the evaluation of potential carcinogenic risks for the human population. We are eager to identify carcinogenic agents before they become recognized human problems. To do the best job possible we must commit ourselves to the intelligent use of the scientific tools that are available and help stimulate the development of tools that may become available in the future. In this way we will make better use of public resources to detect, to assess the risks, and, where necessary, to control the risks of carcinogenic substances.

High on that list of significant developments for the near term and future is the work on biological markers. We can expect that advances in the area of markers for carcinogenesis will help in the development of short-term screening procedures which will allow us to more rapidly, effectively, and efficiently select compounds for more detailed examination and long-term cancer bioassays. These investigations will probe the developmental sequences of carcinogenesis and shed light on potential mechanisms that underlie the development of neoplasms. Undoubtedly, biological markers will also be used more extensively in clinical medicine for a number of different applications.

I believe that science owes the public no less than to bring all of its available methodologies and insights to bear on the pressing, long-standing problem of assessment of tumorgenic responses and the risks which these responses may indicate for humans who are exposed to these agents.

I welcome you to the Conference. The program is bursting with excellent papers and presentations. Your participation, reflective consideration, and dialogue will bring this Conference to a fruitful and productive conclusion.

AN OVERVIEW OF CURRENT RESEARCH EFFORTS ON THE APPLICATION OF BIOLOGICAL MARKERS TO CARCINOGEN TESTING

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The Toxic Substances Control Act (TSCA) was enacted in 1976 on the assumption that: 1) there is substantial exposure of humans and the environment to chemicals; 2) that some of these exposures may present an unreasonable risk of injury; and 3) that existing laws do not fully protect against such injury. The U.S. Environmental Protection Agency (EPA) has a broad range of authorities under this law including screening new chemicals in commerce, requiring industry to test chemicals of concern, and assessing chemicals for control actions.

In carrying out its responsibilities under TSCA, EPA must assess the carcinogenic potential of certain chemicals. For example, examination of a new chemical under Section 5 of TSCA begins with a comprehensive evaluation of any available data on the chemical itself and related analogs. After such an evaluation it may be concluded that the new chemical may have the potential for carcinogenicity but that additional short-term or long-term testing is needed to verify this assumption.

The long-term animal bioassay in rodents is the best available method for detecting carcinogens, however, it is expensive, time consuming, and often provides ambiguous results. Efforts, therefore, are being expended to identify short-term assays with potential application for the identification of chemical carcinogens.

The etiology of cancer is believed to be multifactorial. In a simplified form, it can be envisioned that through the action of chemicals, radiation, viruses, hormones, or other initiating agents, normal cells may be altered. Such "initiated" cells may lie dormant or undergo expression and replication, and form tumors. Nutritional

factors, stress, hormonal imbalance, aging, and the immune system all have been suggested to play an important role in the expression phase of carcinogenesis.

At the biochemical level, we can see that carcinogens, x-rays or alkylators can alter DNA to form DNA-adducts which, if the altered DNA is not repaired, will produce transformed genotypes. These, in turn, will be passed on to daughter cells during cellular replication. With continued cellular proliferation, tumors will be formed.

Biological changes which can be correlated with the carcinogenic process may be used as early indicators or markers of chemical carcinogenesis. For example, four areas of investigation with potential application to short-term testing for carcinogenicity have been identified: 1) tests based on correlating biochemical and immunological changes with the carcinogenic process (tumor markers); 2) tests based on correlating carcinogenicity with the ability of chemicals to cause mutations or to inhibit DNA repair; 3) tests based on the ability of chemicals to transform cells; and 4) tests based on the ability of chemicals to induce benign tumors which correlate with carcinogenicity (limited bioassays).

The search for a blood constituent which is useful as an early indicator of the onset of cancer has attracted the attention of biochemists for some time. This search has not been completely successful because of poor sensitivity of the methods employed and lack of specificity for neoplastic cells. A comprehensive review of the literature on biochemical and immunological markers of carcinogenicity was recently completed. This review identified over 120 markers including alphafetoprotein, CEA, pancreatic glycoprotein, and others. The markers under consideration fell into one of the following ten categories: 1) hepatic and renal enzymes; 2) enzymes of nucleic acid metabolism; 3) carbohydrate metabolizing enzymes; 4) glycotransferases; 5) glycosidases and blood carbohydrates; 6) modified nucleosides of ribonucleic acid; 7) glycoproteins and glycolipids; 8) immunological markers; 9) hormones; and 10) others. The report is now being reviewed by scientists at the Environmental Protection Agency (EPA) and elsewhere for the potential application of tumor markers to carcinogen testing. It is envisioned that following this review, and in conjunction with this symposium, recommendations will be made on the further validation of markers which appear to have potential utility in the identification of chemical carcinogens.

In the area of mutagenesis, the ability of selected mutagenicity and related assay systems to correlate with carcinogenic activity of chemicals is systematically being evaluated. The assays which have been selected for evaluation are based on bacterial and mammalian gene mutation, primary DNA damage, and chromosomal

effects. These evaluations are being conducted by scientists representing academia, industry, and government working through a series of committees to examine the feasibility of using these methods in a pre-chronic testing battery for carcinogenicity.

In the area of cell transformation tests, several systems are currently under consideration. These fall into three basic types: 1) cell strain, those cells with a limited lifespan; 2) cell lines, those cells with an unlimited lifespan; and 3) oncogenic viral-chemical interactions involving cells. These tests are being examined by the appropriate committees of the GENE-TOX program of the EPA for potential use in a screening battery.

In the area of limited bioassays, a comprehensive review of the published literature on limited bioassays yielded over 20 different methods for consideration. Of these, five were selected for additional validation. These are: 1) the Sencar mouse skin tumorigenesis assay; 2) pulmonary tumor induction in strain A mice assay; 3) pulmonary tumor induction in newborn mice assay; 4) mammary tumor induction in female Sprague-Dawley rats assay; and 5) the induction of iron-resistant liver foci assay. When these methods were evaluated for overall accuracy for detecting chemicals which have been shown by the National Cancer Institute, the International Agency for Research on Cancer, or the EPA's Carcinogen Assessment Group to be animal carcinogens, all but the pulmonary tumor induction in strain A mice assay showed potential utility as a pre-screen for the long-term animal bioassay (>86% accuracy in detecting proven carcinogens). Increasing the number of chemicals being examined to include all chemicals judged to be carcinogenic by any investigator reduced the level of accuracy slightly, but again, only the strain A mouse bioassay was found not to be useful.

The utility of these methods in a short-term testing battery for carcinogenicity is still being investigated. Further validation of the methods is necessary before any definitive conclusion could be made.

In summary, biological markers of carcinogenicity may be applied to the investigation of four areas of short-term testing methodologies for carcinogens, namely, tumor markers, mutagenesis, cell transformation, and limited bioassays. I would like to propose that the goals for this symposium on biological markers of carcinogenicity be: 1) to review what is known in this area; 2) to identify areas of research and short-term assays which have potential application to carcinogen testing; and 3) to recommend future directions in research.

CHAIRMAN'S OVERVIEW ON IN VIVO TESTS

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For a number of years, there have been attempts to delineate biological indicators of the progression of the process of carcinogenesis, other than purely morphologic or histopathologic characteristics. Such indicators or "markers" would facilitate following the course of model experiments, might facilitate earlier intervention and treatment in clinical situations, and might be followed as an indicator that there was favorable or unfavorable response to treatment. Within the past decade, the increased emphasis in the area of markers has led to reports of antigenic and enzymic indicators of the presence of tumors. Although further research has not always supported these preliminary results, in other cases it has strengthened the case for the validity of the markers.

The purpose of this symposium is to provide information on advances in the identification and application of useful tumor markers. Coupled with this was the concept that consideration should be given to model tumor systems of relevance to humans, including intestinal and pancreatic cancer. In cancers of these organs, it often is the case that clinical indications of a neoplastic state often are not apparent until the tumor is too far advanced for surgical intervention. Thus, a need certainly exists for identifying markers which will provide a forewarning of the development and growth of a tumor.

The first session will begin with a presentation by Dr. Thomas Hamm, Chemical Industry Institute of Toxicology. Dr. Hamm has also been associated with the National Toxicology Program where he participated in administration of a bioassay program. Dr. Hamm will discuss the lack of specificity of tumor markers in a bioassay

program and why histopathologic examination thus far remains the best diagnostic tool for carcinogenicity studies in animals.

One of the best studied model tumor systems in animals has been the induction of hepatocellular carcinoma in rats. Dr. Emmanuel Farber and associates from the University of Toronto will review the developments in this area and explain why no specific marker for neoplasia has yet been identified in the rat liver system, as well as the basis for their conclusions.

Dr. Daniel S. Longnecker, Dartmouth Medical School, will discuss the experimental rat pancreas model as well as morphologic and biochemical indicators of the neoplastic process in this system.

Human colon cancer represents a continuing problem to the clinician. Within the past 10 or 15 years, reliable and reproducible methods for inducing large bowel neoplasia in rodents have facilitated the identification of the stages of tumor development. Dr. Martin Lipkin, Memorial Sloan-Kettering Cancer Center, has been active in the study of experimentally induced large bowel cancer. In addition, he has applied basic research to the clinical situation, especially in humans with a hereditary predisposition to colorectal cancer. In the same field, that of intestinal cancer, Dr. Bandaru S. Reddy, American Health Foundation, will describe how the results from epidemiologic investigations led to the design and conduct of animal experiments which tend to substantiate the epidemiologic studies. Furthermore, the results of his experiments may point toward feasible dietary modifications for our population.

Skin cancer, although one of the most readily treated and curable forms of cancer, is also one of the most numerous types of tumors. Dr. Margaret L. Kripke, Frederick Cancer Research Facility, has developed a very relevant animal model, namely ultraviolet radiation-induced skin cancers in mice. She will present data on a UV radiation-associated antigen which may lead to interesting advances in this area.