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Genotoxic Effects of Airborne Agents

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FOREWORD

For at least 40 years there has been a great interest in the problems created by infectious airborne agents and other toxic substances transported through the air. During the Second World War, this problem grew out of the very high incidence of upper respiratory infections appearing in new military recruits who were brought together in very large, open quarters. As a result, very interesting methods were developed to measure these airborne agents, especially bacteria, and some important methods were refined for their control. These methods primarily concentrated on ultraviolet radiation, propylene glycol and other means to reduce the dust in an environment. Because of the specialized circumstances at that time the whole consideration of airborne particles became prominent.

Now, with the new strides in the recognition of mutagenic and carcinogenic effects attributed to exposure to airborne chemicals from today's technology, the problem has again become quite prominent. The development of experimental chambers has made it possible to conduct studies under carefully controlled conditions.

For this reason, the organization of this symposium on "Genotoxic Effects of Airborne Agents" is an important milestone in the study of these agents. This symposium brought out many of the new developments and served as an example of the recent, explosive research of environmental mutagenesis and genetic toxicology, of which exposure to airborne chemicals is a very important part in this quickly developing field. The organizers of the symposium and the editors of the symposium proceedings are to be congratulated for having arranged for this important symposium, the proceedings of which serve as a great boost for the study of problems connected with airborne mutagens.

The importance of this field cannot be overemphasized. As a matter of fact, in the area in which I am especially interested, we are considering the quantity and variety of the "mutagen burden" of unavoidable mutagens to which we are regularly exposed. Airborne

chemicals are, of course, a major part of such problems and could have a considerable influence on our health.

Alexander Hollaender

Associated Universities, Inc.

PREFACE

It is generally accepted, but not proven, that exposure to environmental agents is integrally related to the incidence of most cancers. While these agents may also be absorbed or ingested, inhalation is the most common route of exposure in the workplace and in the ambient environment. The recognition of the potentially important role of airborne agents in carcinogenesis has led to the recent development of a number of techniques for specifically assessing the genotoxicity of these agents. Federal legislation, such as the Clean Air Act of 1970 (PL91-604) which specified that inhalants posing a significant carcinogenic risk to man were to be regulated as air pollutants, provided the initial impetus to this research.

The timeliness of a symposium on the genotoxic effects of airborne agents developed from a series of discusions between Dr. Alexander Hollaender and members of the scientific staff of the Medical Department, Brookhaven National Laboratory. This Symposium would emphasize the state-of-the-art techniques for assessing the genotoxicity of airborne agents in general and the current carcinogenic and mutational knowledge of a selected few. Additionally included as appropriate topics for concern were: (i) the monitoring human populations at risk, (ii) the extrapolation of experimental data to human health effects, and (iii) the regulatory aspects of exposure to airborne toxicants.

A program committee consisting of Drs. Robert Drew (Medical Department, Brookhaven National Laboratory), Michael Waters (U.S. Environmental Protection Agency), Frederick de Serres (National Institutes of Environmental Health Sciences), Alexander Hollaender (Associated Universities, Inc.) and chaired by Dr. Raymond Tice (Medical Department, Brookhaven National Laboratory) was formed to evaluate discussion topics and select speakers. The symposium was divided into three sections. The first day would focus on techniques for assessing genotoxicity, techniques which would include the full range of in vitro and in vivo systems. The second day would review and discuss new data on selected inhalants, such as anesthetics, formaldehyde, styrene, radon, nitropyrenes, organic halides, and benzene. Special attention would be given to benzene because it

is one inhalant which had been examined by a number of scientists in different fields and thus could serve as a model airborne agent. The third day would present methods for monitoring human populations. methods for extrapolating experimental data to the human situation and approaches for the regulation of airborne agents.

Any successful symposium and symposium proceedings requires the attention and assistance of many individuals working for a common goal. Special thanks are to be given to Dr. Alexander Hollaender for his guiding touch, to Drs. M. Waters and F. de Serres for their assistance in planning this symposium and for the funds obtained from their respective agencies to support this meeting, to the Session Moderators for thei assistance in obtaining the speakers and in running the program, to the staff of the Medical Department (J. Cutt, K. De Pierro, and T. Smith) and of Brookhaven National Laboratory (D. Schroeder, G. T. Walczyk, H. Boyd, P. Glynn, and others in the housing and travel offices) who did their best to insure a successful meeting. We, the editors of the symposium proceedings, would also like to gratefully acknowledge the prompt attention given to the manuscripts by the contributors. The desire to have a common format for referencing, abbreviations, spelling and presentation necessitated a great many stylistic alterations in some circumstances. We are grateful for the patience of the authors while making these alterations. We are also extremely grateful to the proceedings' secretary, Mrs. Rene Tiernan, for her efforts in completing this book.

Raymond R. Tice
Daniel L. Costa
Karen Schaich

Brookhaven National Laboratory

CONTENTS

SESSION I: ASSAY AND EXPOSURE TECHNOLOGY OF IN VITRO MICROBIAL ASSAY SYSTEMS APPLIED TO AIRBORNE AGENTS

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Scientists involved with bioassaying air samples tend to view air as containing two parts: gases and particles. The papers in this section will include a discussion of the current microbial mutagenesis bioassay methods applicable to these two phases of air.

Atmospheric chemists, however, continue to remind us that the air we breathe is not simply made up of a mixture of gases and particles. They refer to air as a complex, multiphasic system consisting of gases, vapors, and a spectrum of particles ranging in size from 0.01 μm to 100 μm in diameter. Both chemical and physical reactions are known to occur in this dynamic complex aerosol mixture. Future research may open the door to applying microbial bioassays to this complex mixture in a more dynamic exposure system in situ. Current methods, however, limit our discussion to the assay and exposure technology available in the laboratory setting.

The first collection and bioassay of air samples in the 1940's was not performed with microbes but with mice (1). Later, in the 1970's, extracts of urban air were shown to transform rodent cells in culture (2,3). Microbial mutagenesis studies were not reported until a year after Ames described the Salmonella typhimurium plate incorporation assay (4). Three separate groups (5-7) reported the application of this assay to organic extracts of air particles collected by the high volume sampler. The intervening three years have produced a number of studies in this area.

R. H. Jungers' and J. Lewtas's paper describes recent research in methods for collecting and extracting the organics from airborne particles. L. Claxton's paper discusses the biologi-

cal and chemical characterization techniques which can be applied to such samples.

The technical problems associated with collecting sufficient quantitites of artifact-free gas samples from ambient air have prevented significant advances in bioassaying such samples. Advances in chemical identification of many gaseous components of air, however, have stimulated the development of laboratory technology to bioassay individual gases. Early studies (4) employed either desiccators or plastic bags for such exposures. E. Barber's paper reports one of the first efforts to develop an exposure system for the quantitative measurement of the microbial mutagenicity of volatile liquids.

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AN EXPOSURE SYSTEM FOR QUANTITATIVE MEASUREMENTS OF THE MICROBIAL MUTAGENICITY OF VOLATILE LIQUIDS

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It has been estimated that 5×10^{10} grams per day of vapor-phase chemicals and 10^9 grams per day of particulate matter are released into the atmosphere in the United States (1). Since both of these fractions may contribute to the toxic potential of the atmosphere, it seems important to be able to evaluate them in a precise manner.

The standard Salmonella/microsome mutagenicity assay, or Ames test, is a screening test useful in the evaluation of chemicals and mixtures (2). The test has been applied to a variety of environmental samples including food (3-5), beverages (6,7), air (8-10), water (11-13), tobacco (14) and chemicals such as dyes, drugs and pesticides (15-18). The test measures the ability of a chemical or mixture to cause point mutations (reversions) in the test organisms and the results show a reasonably high degree of correlation with known animal carcinogenicity data (15,19-21). In the standard plate incorporation assay, the test plates are prepared at 45 °C and this warm mixture is spread in a very thin layer over the surface of an agar plate (2). The plates are then incubated at 37 °C for two days. This procedure is almost perfectly designed for driving off volatile chemicals and, indeed, this shortcoming has been recognized by many authors (22-30).

To solve this problem we have devised a procedure to assay volatile liquids quantitatively (31). At the heart of our procedure is the incubation vessel depicted in Figure 1. The containment system is composed of Pyrex, Teflon, stainless steel and Viton components. Therefore, it is autoclaveable and highly inert toward many chemicals. The procedure we use is as follows: First, the Ames test plates are prepared exactly as described in