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*Genotoxic
Effects of
Airborne
Agents*

Edited by
RAYMOND R. TICE
DANIEL L. COSTA
KAREN M. SCHAICH

Genotoxic Effects of Airborne Agents

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RAYMOND R. TICE

DANIEL L. COSTA

and

KAREN M. SCHAICH

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***Genotoxic
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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 discussions 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 their 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

SECTION A: ASSAY AND EXPOSURE TECHNOLOGY

Session I: Assay and Exposure Technology of In Vitro Microbial Assay Systems Applied to Airborne Agents	
Joellen Lewtas, Moderator.....	1
An Exposure System for Quantitative Measurements of the Microbial Mutagenicity of Volatile Liquids	
Eugene D. Barber and William H. Donish.....	3
Review of Fractionation and Bioassay Characterization Techniques for the Evaluation of Organics Associated with Ambient Air Particles	
Larry D. Claxton.....	19
Airborne Particle Collection and Extraction Methods Applicable to Genetic Bioassays	
Robert H. Jungers and Joellen Lewtas.....	35
Session II: Assay and Exposure Technology of In Vitro Mammalian Cell Systems Applied to Airborne Agents	
Donald E. Rounds, Moderator.....	49
In Vitro Analysis of Mammalian Cells Exposed In Vitro and In Vivo to Airborne Agents	
R. R. Guerrero and D. E. Rounds.....	51
Methods for Detecting Gaseous and Volatile Carcinogens Using Cell Transformation Assays	
G. G. Hatch, P. D. Mamay, M. L. Ayer, B. C. Castro and S. Nesnow.....	75
CHO/HGPRT Mutation Assay: Evaluation of Gases and Volatile Liquids	
David F. Krahn, Francis C. Barsky and Kevin T. McCooley.....	91

Lung Cells Grown on Cellulose Membrane Filters as an In Vitro Model of the Respiratory Epithelium Ronald E. Rasmussen and T. Timothy Crocker.....	105
Session III: Plant Bioassays for the Detection of Airborne Genotoxic Agents L. A. Schairer, Moderator.....	121
Monitoring Ambient Air for Mutagenicity Using the Higher Plant <i>Tradescantia</i> L. A. Schairer, R. C. Sautkulis and N. R. Tempel.....	123
Environmental Clastogens Detected By Meiotic Pollen Mother Cells Te-Hsiu Ma, Van A. Anderson and Iftikharuddin Ahmed.....	141
Plant Genetic Systems with Potential for the Detection of Atmospheric Mutagens Milton J. Constantín.....	159
Session IV: Animal Exposure Technology for the Detection of Genotoxic Agents Robert T. Drew, Moderator.....	179
Systems for Exposure of Animals to Airborne Agents Robert T. Drew.....	181
Techniques for the Generation and Monitoring of Vapors Gary O. Nelson.....	197
Problems Associated with Assessing the Mutagenicity of Inhalable Particulate Matter Otto G. Raabe.....	209
SECTION B: AIRBORNE AGENTS	
Session V: Benzene R. Snyder, Moderator	
An Overview of the Problem of Benzene Toxicity and Some Recent Data on the relationship of Benzene Metabolism to Benzene Toxicity R. Snyder, D. Semmett, C. Witmer and J. J. Kocsis.....	225

Benzene Metabolites: Evidence for an Epigenetic Mechanism of Toxicity R. D. Irons and R. W. Pfeifer.....	241
Cytogenetic Effects of Inhaled Benzene in Murine Bone Marrow R. R. Tice, T. F. Vogt and D. L. Costa.....	257
Benzene Leukemogenesis Bernard D. Goldstein and Carroll A. Snyder.....	277
Session VI: Organic Halides C. Maltoni, Moderator	
Genetic Effects of Ethylene Dibromide in <i>Drosophila Melanogaster</i> P. Kale and J. W. Baum.....	291
Mutagenic and Oncogenic Effects of Chloromethanes, Chloroethanes and Halogenated Analogues of Vinyl Chloride Peter F. Infante and Theodora A. Tsongas.....	301
Vinyl Chloride: A Model Carcinogen for Risk Assessment Cesare Maltoni, Giuseppe Lefemine, Adriano Ciliberti, Guiliano Cotti and Donato Carretti.....	329
Session VII: Miscellaneous Agents M. K. Conner and J. W. Allen, Moderators	
Inhalation Anesthetics Vincent F. Simmon.....	345
Mutagenic and Carcinogenic Effects of Formaldehyde Craig J. Boreiko, David B. Couch and James A. Swenberg.....	353
The Microbial Mutagenicity of Nitroarenes Robert Mermelstein, Herbert S. Rosenkranz and Elena C. McCoy.....	369
Mutagenic Effects in Human and Mouse Cells by a Nitropyrene C. F. Arlett, J. Cole, B.C. Broughton, J. Lowe and B. A. Bridges.....	397

Genotoxic Properties of Radon
and Its Daughters

Naomi H. Harley, Stuart M. Altman
and Bernard S. Pasternack..... 411

Multiple Tissue Comparisons of Sister Chromatid
Exchanges Induced by Inhaled Styrene

M. K. Conner, V. Alarie and
R. L. Dombroske..... 433

An Overview of Ethyl Carbamate (Urethane)
and Its Genotoxic Activity

James W. Allen, Yousuf Sharief and
Robert J. Langenbach..... 443

SECTION C: MONITORING AND RISK ASSESSMENT

Session VIII: Monitoring of Human Populations At Risk

R. R. Tice, Moderator..... 461

Cytogenetic Monitoring of Human Populations

Sheila M. Galloway and Raymond R. Tice..... 463

Thioguanine Resistent Lymphocytes As
Indicators of Somatic Cell Mutation
in Man

Richard J. Albertini and David L. Sylwester..... 489

Detection of Point Mutations in
Mammalian Sperm

H. V. Malling, J. G. Burkhart,
M. A. Baig and A. A. Ansari..... 507

Session IX: Extrapolation of Genotoxic Data
To Human Health Effects

V. P. Bond, Moderator..... 519

Extrapolation of Laboratory Data
to Human Health Effects

David Hoel..... 521

Molecular Dosimetry as a Bridge Between
Mammalian and Non-Mammalian Test Systems

William R. Lee..... 527

Is Radiation an Appropriate Model for
Chemical Mutagenesis and Carcinogenesis?

V. P. Bond..... 539

Session X: Regulatory Risk Assessment	
D. C. Borg, Moderator.....	553
Impact of Energy and Pollution on Public Health	
Samuel C. Morris, III.....	555
Policy and Procedures for Using Mutagenicity Data in Assessing Genetic Risk	
Peter Voytek.....	579
The Sensitivity of Method Procedure as a Regulatory Mechanism for Approval of Carcinogens	
Theodore M. Farber.....	589

CONTRIBUTED PAPERS

The Human Genetic Risk of Airborne Genotoxics: An Approach Based on Electrophoretic Techniques Applied to Mice	
F. M. Johnson and Susan E. Lewis.....	595
Photon-Emitting Microorganisms as Test Objects for Detecting Genotoxic Agents	
Stanley Scher and Richard A. Wecher.....	607
Detection of Genotoxic Airborne Chemicals in Rat Liver Culture Systems	
S. Ved Brat, C. Tong, and G. M. Williams.....	619
The Induction of Sister Chromatid Exchange in Human Lymphocytes and Bacterial Mutagenesis by Organic Extracts of Urban Airborne Particles	
J. M. Lockard, C. J. Viau, C. Lee-Stevens, J. C. Caldwell, J. P. Wojciechowski, H. G. Enoch and P. S. Sabharwal.....	633
An epidemiological Study of Cancer Deaths in Pathfor, Kentucky	
Samuel G. Gregorio.....	635
LIST OF PARTICIPANTS.....	643
AGENT INDEX.....	651
INDEX.....	655

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

Joellen Lewtas, Moderator

U.S. Environmental Protection Agency
Research Triangle Park, NC 27711 (U.S.A.)

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 quantities 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

Eugene D. Barber and William H. Donish

Eastman Kodak Co., Rochester, NY 14650 (U.S.A.)

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