

CHEMICAL MUTAGENS

**Principles and Methods
for Their Detection**

Volume 3

Edited by A. Hollaender

Sponsored by the Environmental Mutagen Society

(1) **CHEMICAL MUTAGENS**

Principles and Methods for Their Detection

Volume 3

(2) **Edited by Alexander Hollaender**

Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee
and
University of Tennessee, Knoxville

with the cooperation of
**ERNST FREESE, KURT HIRSCHHORN,
AND MARVIN LEGATOR**



PLENUM PRESS • NEW YORK-LONDON • 1973

First Printing - August 1973
Second Printing - August 1977

Library of Congress Catalog Card Number 73-128505
ISBN 0-306-37103-0

© 1973 Plenum Press, New York
A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

United Kingdom edition published by Plenum Press, London
A Division of Plenum Publishing Company, Ltd.
Davis House (4th Floor), 8 Scrubs Lane, Harlesden, London, NW10 6 SE, England

All rights reserved

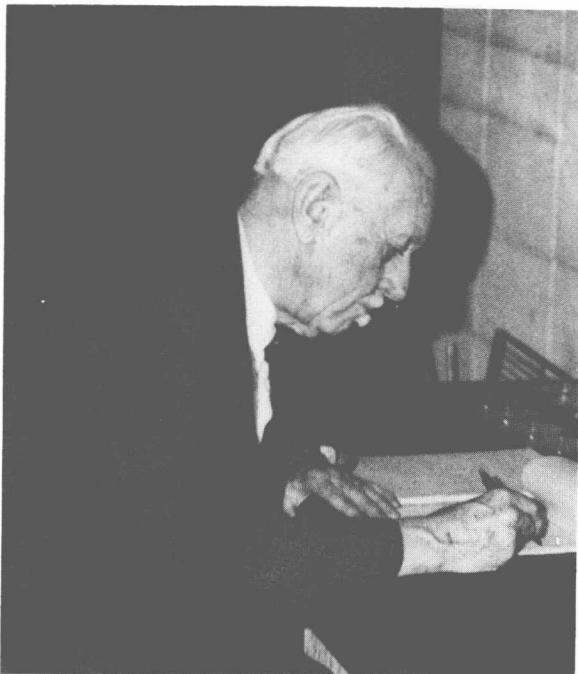
No part of this publication may be reproduced in any
form without written permission from the publisher

Printed in the United States of America

CHEMICAL MUTAGENS

Principles and Methods for Their Detection

Volume 3



*Dedicated to Professor Karl Sax
on the Occasion of His Eightieth Birthday*

Professor Sax has long been recognized as the founder of the American school of experimental chromosome cytology. With his students and his wife, Hally Sax, he laid the foundations for much of our present knowledge concerning the effects of radiation and chemicals on the induction of chromosome aberrations. In a more fundamental sense, he employed these agents as tools to investigate chromosome structure and function. His interests and influence have not been limited to purely academic problems but have extended to relevant social questions as well. Before most scientists and the public were aware of the hazards of certain drugs and food additives, Professor Sax was studying their effects on cells, and long before the widespread concern with overpopulation, Professor Sax was warning us through his writing and lecturing of the dangers that lay ahead. It is hoped that Karl and Hally Sax will continue for many years to explore the intricacies of chromosomes with that special combination of insight, zest, and grace that is their trademark.

Preface

The ready acceptance and wide demand for copies of the first two volumes of *Chemical Mutagens: Principles and Methods for Their Detection* have demonstrated the need for wider dissemination of information on this timely and urgent subject. Therefore, it was imperative that a third volume be prepared to include more detailed discussions on techniques of some of the methods that were presented from a theoretical point of view in the first two volumes, and to update this rapidly expanding field with current findings and the new developments that have taken place in the past three years. Also included is a special chapter by Dr. Charlotte Auerbach giving the historical background of the discovery of chemical mutagenesis.

Methods for recognizing mutagenic compounds *in vitro* are a necessary preliminary step toward arriving at satisfactory solutions for recognizing significant mutation rates in man, which must be done before our test-tube methods of detection can be considered reliable. Two chapters in this volume make important contributions to this problem.

Due to the increasing activity in efforts to perfect techniques for detecting chemical mutagens and their effects on man, it is planned to continue this series of volumes as necessary to keep abreast of current findings.

Alexander Hollaender

*Biology Division, Oak Ridge
National Laboratory and
The University of Tennessee*

Contributors to Volume 3

N. G. Anderson

Molecular Anatomy Program
Oak Ridge National Laboratory
Oak Ridge, Tennessee

C. Auerbach

Department of Genetics
The University
Edinburgh, Scotland

Alexej B. Boškovec

Insect Chemosterilants Laboratory
Beltsville, Maryland

Donald Clive

Mutagenesis Branch
National Institute of Environmental
Health Sciences
National Institutes of Health
Research Triangle Park, North Carolina

G. E. Cosgrove

Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

W. Gary Flamm

Mutagenesis Branch
National Institute of Environmental
Health Sciences
National Institutes of Health
Research Triangle Park, North Carolina

W. M. Generoso

Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Alain Léonard

Laboratory of Genetics
Department of Radiobiology
C.E.N./S.C.K., Belgium

J. V. Neel

The University of Michigan Medical
School
Ann Arbor, Michigan

Howard B. Newcombe

Biology and Health Physics Division
Atomic Energy of Canada Limited
Chalk River, Ontario, Canada

James B. Patterson

Mutagenesis Branch
National Institute of Environmental
Health Sciences
National Institutes of Health
Research Triangle Park, North Carolina

James D. Regan

Carcinogenesis Program
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

L. A. Schairer
Biology Department
Brookhaven National Laboratory
Upton, New York

R. B. Setlow
Carcinogenesis Program
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

A. H. Sparrow
Biology Department
Brookhaven National Laboratory
Upton, New York

T. O. Tiffany
Molecular Anatomy Program
Oak Ridge National Laboratory
Oak Ridge, Tennessee

A. G. Underbrink
Biology Department
Brookhaven National Laboratory
Upton, New York

John S. Wassom
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Friedrich K. Zimmermann
Department of Biology
Brooklyn College of The City University
of New York
Brooklyn, New York

Contents of Volume 3

| | |
|-----------------------------------|-----|
| Contents of Volume 1 | xix |
| Contents of Volume 2 | xx |

Chapter 24

| | |
|--|---|
| History of Research on Chemical Mutagenesis | 1 |
| by C. Auerbach | |

| | |
|---|----|
| I. Introduction | 1 |
| II. War Gases | 2 |
| III. Other Alkylating Agents | 5 |
| IV. Nitroso Compounds | 7 |
| V. Urethane | 8 |
| VI. Alkaloids | 9 |
| VII. Inorganic Salts | 9 |
| VIII. Formaldehyde, Organic Peroxides | 10 |
| IX. Nitrous Acid | 11 |
| X. Calf Thymus DNA and Other Macromolecules, Carcinogens | 12 |
| XI. Phenols | 13 |
| XII. Basic Dyes | 14 |
| XIII. Purines | 15 |
| XIV. Base Analogues | 16 |
| XV. Epilogue | 18 |
| XVI. References | 19 |

*Chapter 25***Observations on Meiotic Chromosomes of the Male Mouse****as a Test of the Potential Mutagenicity of Chemicals****in Mammals** 21

by Alain Léonard

| | | |
|------|---|----|
| I. | Introduction | 21 |
| II. | Methods for Detecting Chromosome Aberrations | 22 |
| | A. Spermatogenesis in the Mouse | 22 |
| | B. Description of Methods | 24 |
| | C. Techniques for Preparation of Meiotic Chromosomes | 29 |
| III. | Experimental Results with Ionizing Radiation | 34 |
| | A. Spermatocyte Test on Treated Males | 34 |
| | B. F ₁ Translocation Test | 35 |
| IV. | Experimental Results with Chemical Mutagens | 38 |
| | A. Review of Results | 38 |
| | B. Detailed Results | 41 |
| | C. Evaluation of Results and Prospects of the Experimental Approach | 48 |
| V. | Acknowledgments | 48 |
| VI. | References | 49 |

*Chapter 26***Techniques for Monitoring and Assessing the Significance****of Mutagenesis in Human Populations** 57

by Howard B. Newcombe

| | | |
|-------|---|----|
| I. | Introduction | 57 |
| II. | Kinds of Information about Human Populations Needed for Monitoring | 58 |
| III. | Limitations and Advantages of Using Existing Population Records | 60 |
| IV. | Information Content of the Available Records | 61 |
| V. | Methods of Record Linkage | 63 |
| VI. | Relevant Data Obtained by Record Linkage A. Amount of Hereditary Disease | 65 |
| | B. Distinguishing the Monomeric and Multifactorial Parts | 66 |
| | C. Tests for a Mutation-Maintained Component | 70 |
| | D. Tests for a Selection-Maintained Component | 72 |
| VII. | Future Accessibility of Diagnostic Data | 73 |
| VIII. | Mutagenesis and the Search for an Optimum Environment for Man | 74 |
| IX. | Conclusions for Geneticists | 75 |
| X. | References | 75 |

*Chapter 27***Specific-Locus Mutational Assay Systems for Mouse**

| | |
|---|-----|
| Lymphoma Cells | 79 |
| by Donald Clive, W. Gary Flamm, and James B. Patterson | |
| I. Introduction | 79 |
| II. HGPRT Locus | 80 |
| III. TK Locus: General Principles | 84 |
| IV. TK Locus in a Mutational Assay System | 86 |
| V. Concluding Remarks | 93 |
| VI. Acknowledgments | 94 |
| Appendix I: Cell Material and Techniques | 95 |
| A. Cells | 95 |
| B. Media (Nonselective Suspension—Growth and Cloning) | 95 |
| C. Mutant Stocks | 95 |
| D. Selective Media | 95 |
| E. Thymidine Kinase Assay | 96 |
| F. Spontaneous Mutation Rates | 96 |
| G. Maintaining Cultures with Low Mutant Background | 96 |
| H. Mutagen Assay (Forward) | 98 |
| I. Mutagen Assay (Reverse) | 100 |
| Appendix II: The <i>Mycoplasma</i> Menace | 100 |
| VII. References | 102 |

*Chapter 28***Approaches to Monitoring Human Populations for Mutation**

| | |
|--|-----|
| Rates and Genetic Disease | 105 |
| by J. V. Neel, T. O. Tiffany, and N. G. Anderson | |
| I. Introduction | 105 |
| II. Theoretical Importance of Measurements in Man | 106 |
| III. Practical Importance of Human Genetic Monitoring | 109 |
| IV. Study of Mutation at the Molecular Level | 110 |
| V. Critique of Genetic Monitoring Based on Electrophoresis | 113 |
| VI. Does the Initial Approach Meet the Criteria for a Satisfactory Monitoring System? | 116 |
| VII. Estimates of the Magnitude of Effort Required | 117 |
| VIII. Importance of Accurate Information | 119 |
| IX. Some Technological Requirements | 120 |
| X. Assessment of Technological Possibilities (Using Electrophoresis) | 123 |
| A. Sample Collection and Preparation | 123 |
| B. Electrophoretic Separation | 125 |
| C. Detection Using Immunological Methods | 128 |

| | | |
|-------|--|-----|
| XI. | Methods Based on Activity Measurements | 131 |
| A. | Enzyme Variant Scanning | 131 |
| B. | Isozyme Scanning | 132 |
| C. | Enzyme Group Evaluation | 135 |
| XII. | Low Molecular Weight Substances | 136 |
| A. | Scanning for Screen Evaluation | 136 |
| B. | Automation of Screening Methods for Low Molecular Weight Substances | 139 |
| XIII. | Confirmatory Analyses, Genetic Follow-up, and Data Reduction | 140 |
| XIV. | Genetic Monitoring as a National Problem | 141 |
| A. | Cost/Benefit Considerations—Does the Potential Total Benefit Exceed the Total Cost? | 142 |
| B. | Benefits to Patients | 143 |
| C. | Benefits to Society | 144 |
| D. | Benefits to Research, Medical, and Decision-Making Communities | 145 |
| E. | Ethical Considerations | 147 |
| XV. | Conclusions | 147 |
| XVI. | References | 147 |

Chapter 29

| | |
|---|-----|
| Repair of Chemical Damage to Human DNA | 151 |
| by James D. Regan and R. B. Setlow | |

| | | |
|------|---|-----|
| I. | Introduction | 151 |
| II. | Sequence of Molecular Events in Experiments with Mutagenic and Carcinogenic Agents | 152 |
| A. | Typical Test Systems | 152 |
| B. | Introduction of the Agent | 152 |
| C. | Effect of Serum in the Treatment Medium | 152 |
| D. | Active and Nonactive Forms of Agents | 153 |
| E. | Local Dose to the Cellular DNA | 153 |
| F. | Induction and Repair of DNA Lesions | 153 |
| G. | Residual (Unrepaired) Lesions | 154 |
| III. | What Can DNA Repair Studies Tell Us? | 154 |
| A. | Extent of Repair | 154 |
| B. | Nature of the Repair Event | 154 |
| C. | Nature of the Lesion | 155 |
| IV. | Methods of Studying Repair | 155 |
| A. | Thymidine Uptake | 155 |
| B. | Unscheduled Synthesis | 155 |
| C. | Repair Replication | 156 |

| | |
|---|-----|
| D. Excision of UV-Induced Pyrimidine Dimers | 157 |
| E. Photolysis of 5-Bromodeoxyuridine | 158 |
| V. The Two Forms of Repair as Measured by BrUra | |
| Photolysis | 159 |
| A. Ionizing-Type Repair | 159 |
| B. UV-Type Repair | 161 |
| VI. Classification of DNA-Damaging Chemical Agents | |
| According to the Repair Sequence Induced | 162 |
| A. Ionizing-Type Repair after Chemical Damage | 162 |
| B. UV-Type Repair after Chemical Damage | 164 |
| VII. Xeroderma Pigmentosum and UV-Type Repair after | |
| Chemical Damage to DNA | 165 |
| VIII. Summary of Present Interpretations | 167 |
| IX. Acknowledgments | 169 |
| X. References | 169 |

*Chapter 30***Tradescantia Stamen Hairs: A Radiobiological Test System****Applicable to Chemical Mutagenesis** 171

by A. G. Underbrink, L. A. Schairer, and A. H. Sparrow

| | |
|--|-----|
| I. Introduction | 171 |
| II. Techniques and Procedures | 177 |
| A. Cultivation of Plants | 177 |
| B. Screening the Plants | 178 |
| C. Selection and Maintenance of Cuttings for | |
| Experimental Purposes | 179 |
| D. The Flower | 181 |
| E. Flower Production Records | 182 |
| F. Numbers of Cuttings and Stamens Required | 184 |
| G. Handling the Cuttings during Experimentation | 188 |
| H. Types of Aberrations Found in Stamen Hairs | 189 |
| I. Definition of a Mutant or Aberrant Event | 194 |
| J. Loss of Reproductive Integrity (Stamen-Hair Stunting) | 195 |
| K. Preparation of Materials for Scoring | 196 |
| L. Scoring Procedures | 197 |
| III. Characteristic Data Obtained from the Stamen-Hair | |
| System | 198 |
| IV. Summary | 202 |
| V. Acknowledgments | 203 |
| VI. References | 204 |

Chapter 31

| | |
|--|-----|
| Detection of Genetically Active Chemicals Using Various Yeast Systems | 209 |
| by Friedrich K. Zimmermann | |
| I. Brief Description of the Yeasts Used in Genetic Research | 209 |
| II. Techniques | 210 |
| A. Media | 210 |
| B. Culturing Yeast and Genetic Analysis | 213 |
| C. Preparations of Synchronous Cultures | 215 |
| D. Maintenance of Stock Cultures | 215 |
| III. Treatment Conditions | 216 |
| A. Treatment in Suspension | 216 |
| B. Spot Test on Plates | 217 |
| IV. Systems Used to Detect Mutagenicity of Chemicals | 220 |
| A. Forward Mutation | 220 |
| B. Reverse Mutation | 225 |
| C. Mutagenesis in Meiotic Cells | 226 |
| D. Cytoplasmic Inheritance | 227 |
| V. Evaluation of Mutagenesis Experiments and Complications | 229 |
| A. Mutation Fixation and Mutation Expression | 229 |
| B. Dose-Response Curves | 231 |
| VI. The Problem of Metabolic Activation | 232 |
| VII. Mitotic Recombination | 233 |
| A. Mitotic Crossing-Over | 233 |
| B. Mitotic Gene Conversion | 234 |
| VIII. Methods Used to Increase Sensitivity to Genetically Active Agents | 235 |
| IX. General Evaluation | 236 |
| X. Acknowledgments | 237 |
| XI. References | 237 |

Chapter 32

| | |
|--|-----|
| Total Reproductive Capacity in Female Mice: Chemical Effects and Their Analysis | 241 |
| by W. M. Generoso and G. E. Cosgrove | |
| I. Introduction | 241 |
| II. Oocyte Development and Responses to Radiation Effects | 242 |
| III. General Procedure | 243 |
| IV. Fertility Effects of Alkylating Chemicals on Female Mice | 249 |
| V. Delayed Pathological and Survival Effects in Chemically Treated Female Mice in the Total Reproductive Capacity Experiment | 252 |

| | | |
|-------|--|-----|
| VI. | Importance of Using Females in Fertility Studies | 254 |
| VII. | Need for Assay Systems in Female Mice to Measure Induced Heritable Genetic Damage | 256 |
| VIII. | References | 257 |

Chapter 33

| | |
|---|-----|
| Insect Chemosterilants as Mutagens | 259 |
| by Alexej B. Bořkovec | |

| | | |
|------|---|-----|
| I. | Introduction | 259 |
| II. | Screening of Chemosterilants | 261 |
| III. | Classification of Chemosterilants | 262 |
| IV. | Conclusion | 267 |
| V. | References | 268 |

Chapter 34

| | |
|---|-----|
| The Literature of Chemical Mutagenesis | 271 |
| by John S. Wassom | |

| | | |
|------|---|-----|
| I. | Introduction | 271 |
| A. | Restatement of an Old Problem | 271 |
| B. | Chemical Mutagenesis and Its Literature | 271 |
| II. | The State of Chemical Mutagenesis Literature | 276 |
| III. | Sources and Types of Chemical Mutagenesis Literature .. | 277 |
| IV. | Organization of Chemical Mutagenesis Information | 281 |
| A. | General Methods | 281 |
| B. | Specialized Methods | 282 |
| V. | Some Suggestions for Improving Literature Control | 285 |
| VI. | Conclusion | 286 |
| VII. | Acknowledgments | 287 |

| | |
|----------------------------|-----|
| Author Index | 289 |
| Subject Index | 297 |

Contents of Volume 1

Chapter 1

| | |
|---|---|
| Molecular Mechanisms of Mutations..... | 1 |
| by Ernst Freese | |

Chapter 2

| | |
|---|----|
| Correlation Between Teratogenic and Mutagenic Effects of Chemicals in Mammals..... | 57 |
| by Harold Kalter | |

Chapter 3

| | |
|--|----|
| The Mutagenicity of Chemical Carcinogens: Correlations, Problems, and Interpretations | 83 |
| by Elizabeth C. Miller and James A. Miller | |

Chapter 4

| | |
|---|-----|
| Effects of DNA: Chemical Methods | 121 |
| by P. Brookes and P. D. Lawley | |

Chapter 5

| | |
|---|-----|
| Physical-Chemical Methods of the Detection of the Effect of Mutagens on DNA..... | 145 |
| by Bernard S. Strauss | |

Chapter 6

| | |
|---|-----|
| Effects on DNA: Transforming Principle | 175 |
| by Roger M. Herriott | |

Chapter 7

- Mutagen Screening with Virulent Bacteriophages** 219
by John W. Drake

Chapter 8

- Prophage Induction in Lysogenic Bacteria as a Method of Detecting Potential Mutagenic, Carcinogenic, Carcinostatic, and Teratogenic Agents** 235
by Bernard Heinemann

Chapter 9

- The Detection of Chemical Mutagens with Enteric Bacteria** 267
by Bruce N. Ames

Addendum to Chapter 9

- Mutagenesis Studies with *Escherichia coli* Mutants with Known Amino Acid (and Base-Pair) Changes** 283
by C. Yanofsky

Chapter 10

- Mutation Induction in Yeast** 289
by R. K. Mortimer and T. R. Manney

- Author Index** xxix
Subject Index xlix