PLEASE DON'T FORGET YOUR PROGRAM
AND ABSTRACT BOOKLET

We're sorry—but extra copies of either will cost $2.50.

— NOTES —

1. Papers are to be read by the underscored author if other than the first named.

2. Abstracts were not requested for symposia.

3. EMS badges will be honored for the Environmental Mutagen Society/Society of Toxicology combined sessions, Sessions (Ea) and (Fa).
SEVENTH ANNUAL MEETING

ENVIRONMENTAL MUTAGEN SOCIETY

THE ENVIRONMENTAL MUTAGEN SOCIETY was founded in 1969 and incorporated under the laws of the District of Columbia. It is operated to encourage the study of mutagens in the human environment—particularly as they may affect public health—and to engage in sponsor research, study, and dissemination of information related to this problem.

OFFICERS OF THE SOCIETY

President - F. J. DE SERRES, National Institute of Environmental Health Sciences (1976)

Vice-President - J. W. DRAKE, University of Illinois (Urbana) (1976)

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Treasurer - V. A. RAY, Pfizer Pharmaceuticals (1977)

Administrative Officer

RICHARD J. BURK, JR., 4720 Montgomery Lane, Suite 506
Bethesda, MD. (Telephone: 301/654-3080)
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MARVIN S. LEGATOR (1976)          ERROL ZEIGER (1978)

PROGRAM COMMITTEE

J. W. DRAKE, Chairman

REGISTRATION FEES AND HOURS

Fees:        Member $20.00
             Non-Member $25.00
             Student $5.00
             Spouse $2.00

Hours:       Friday, March 12 9:00 a.m. - 7:00 p.m.
             Saturday, March 13 8:00 a.m. - 5:00 p.m.
             Sunday, March 14  9:00 a.m. - 5:00 p.m.
             Monday, March 15  8:00 a.m. - NOON

Convention Lobby, Marriott Motor Hotel

GENERAL INFORMATION

The 7th Annual Meeting of the Environmental Mutagen Society will be held at the Marriott Motor Hotel, Atlanta, Georgia, 12-15 March 1976. Airport limousine service is available from the Atlanta Airport (@ 15 minutes) to the hotel. Miss Barbara A. Coberly, Associate Administrator, may be contacted at the registration desk for any special assistance needed.

LOCAL COMMITTEE

Dennis Smith, Chairman
9:30 a.m.  (Aa-1) N-Hydroxy-2-Aminofluorene is the Principal Mutagen Produced from 2-Acetylaminofluorene.  
D. L. Stout*, J. N. Baptist*, T. S. Matney, and C. R. Shaw 
The University of Texas System Cancer Center and Health 
Science Center

9:45 a.m.  (Aa-2) 5-Halogenated Uracil Base Analog Mutagenesis. 
M. F. Walton* and R. B. Cumming* 
Oak Ridge National Laboratory

10:00 a.m.  (Aa-3) Mutagenicity of Compounds Structurally Related to 
ICR-170 and ICR-191 in the Presence of O₂ or N₂ in 
Neurospora crassa.  
H. E. Brockman and W-Z. Whong* 
Illinois State University

10:15 a.m.  (Aa-4) Tester Strains for the Identification of Specific 
Base Pair Substitution Mutations in Bacteriophage T4. 
L. S. Ripley* 
University of Illinois, Urbana

10:30 a.m.  (Aa-5) Characterizations of Yeast Mutants Defective in 
Iso-1-Cytochrome a: Studies of Mutagenic Specificities. 
F. Sherman and S. Consaul* 
University of Rochester School of Medicine and Dentistry

10:45 a.m.  (Aa-6) Linearity of Induced Mutagenesis at Low UV Doses 
in Yeast.  
F. Eckardt* and R. H. Haynes 
York University, Canada

11:00 a.m.  (Aa-7) Non-Genetic Factors Affecting the Quantitative 
Repair Capability of Cells.  
P. Karran*, A. Moscona*, A. Norin*, D. Scudiero*, and 
B. Strauss 
University of Chicago

11:15 a.m.  (Aa-8) Interspecific Cytogenetics: The Quantitative Effect 
of Interphase Chromosome Disposition. 
P. Clifford* 
University of California, Berkeley
FRIDAY

(Aa) FUNDAMENTAL MECHANISMS (continued)

11:30 a.m.  (Aa-9) Some Useful Equations for Determining Sample Size in Spontaneous and Induced Mutation Experiments where the Standard Deviation is Proportional to the Mean. K. H. Thompson* and R. C. Sparrow* Brookhaven National Laboratory

11:45 a.m.  (Aa-10) Reversibility of the Inhibitory Effects of Hexachlorophene and Decanoic Acid in Bacillus subtilis. B. C. Levin* and E. Freese National Institutes of Health

12:00 p.m. (Aa-11) Effect of Excision Repair on Azide Induced Mutagenesis. A. Kleinhoff*, J. A. Smith* and R. A. Nilan Washington State University, Pullman

- NOTES -
FRIDAY
South Grand Ballroom
9:30 a.m.

(Ab) SYSTEM DEVELOPMENT: MICROBIAL AND MAMMALIAN

Chairman: K. C. Bora
Environmental Health
Directorate, Canada

Co-Chairman: E. Zeiger
Food & Drug Administration

9:30 a.m.  /  (Ab-1) Use of the Spot Test for the Detection of Mutagenic Activities of Environmental Agents in Neurospora crassa.
T-M. Ong
National Institute of Environmental Health Sciences

9:45 a.m.  /  (Ab-2) Inactivation and Mutation Induction by ICR-170 in UV-Sensitive Strains of Neurospora crassa.
H. Inoue*, T. Ong and F. J. de Serres
National Institute of Environmental Health Sciences

10:00 a.m.  /  (Ab-3) Comparative Studies of Six Compounds in Saccharomyces cerevisiae and Salmonella typhimurium Reversion Systems.
M. M. Shahin and R. C. von Borstel
University of Alberta, Canada

10:15 a.m.  /  (Ab-4) Detection of Mutagen-Induced Genetic Duplications in Salmonella typhimurium.
G. R. Hoffmann and R. W. Morgan*
Meredith College

10:30 a.m.  /  (Ab-5) The Mutagenicity of Heterocyclic N-Nitrosoamines for Salmonella typhimurium.
E. Zeiger and A. T. Sheldon*
Food and Drug Administration, Washington, D.C.

10:45 a.m.  /  (Ab-6) Biotransformation Capacity of Extracts from Rabbit Testis-Tissue Using Microorganisms as Indicators.
P. Maier* and H. V. Malling
National Institute of Environmental Health Sciences

11:00 a.m.  /  (Ab-7) The Use of Trimethylphosphate (TMP) as a Positive Control Agent. II. The Salmonella/microsome Test; the Salmonella/urine Assay and the Micronucleus Test.
M. G. Farrow, J. Barnett*, L. J. Lassen* and L. Wetzel*
Wyeth Laboratories

11:15 a.m.  /  (Ab-8) A Modified Host-Mediated Assay Using Human Cells.
C. C. Huang
Roswell Park Memorial Institute
FRIDAY

(Ab) SYSTEM DEVELOPMENT: MICROBIAL AND MAMMALIAN (continued)

11:30 a.m.  (Ab-9) Development of an in vivo Mammalian Microlesion Detection System.
            C. Kane*, J. B. Mays* and J. E. McAninch*
            National Center for Toxicological Research

11:45 a.m.  (Ab-10) Problems in the Evaluation of Human Safety from Genetic Hazards of Environmental Chemicals.
            K. C. Bora
            Environmental Health Directorate, Canada

12:00 n.   (Ab-11) Deuterium Isotope Effects in Mutagenesis
            by Nitrosamine Compounds
            - NOTES -
            Rosaline K. Elispru
FRIDAY
North Grand Ballroom
2:00 p.m.

(Ba) SYMPOSIUM: MUTAGENIC MECHANISMS IN EU CARYOTES

Chairwoman: L. Prakash
University of Rochester

(Ba-1) Repair Capacity and Mutagenesis in Yeast.
L. Prakash
University of Rochester

(Ba-2) Isolation and Characterization of Mutants of Mammalian Somatic Cells.
L. Siminovitch
University of Toronto, Canada

(Ba-3) A Search for Mitotic Recombination in Cultured Mammalian Cells.
L. A. Chasin
Columbia University

- NOTES -
FRIDAY

8:00 p.m. WELCOME F. J. de Serres
President
Tara Ballroom 1-3

8:00 p.m. RECEPTION Tara Ballroom 1-3

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SATURDAY, MARCH 13, 1976
Center Grand Ballroom
9:00 a.m.

(Ca) SUSPECT COMPOUNDS

Chairman:  W. R. Lower
University of Missouri

Co-Chairwoman:  F. Hollingsworth
Georgia Mental Health Institute

9:00 a.m.  
F. Hollingsworth*, D. A. Shafer* and A. Falek
Georgia Mental Health Institute and Emory University

9:15 a.m.  
(Ca-2) Cytogenetic Effects of In Vivo Administration of Methadone and L-Alpha-Acetyl Methadone (LAAM).
S. S. Matsuyama, V. Charuvastra*, L. F. Jarvik, T-K. Fu,
K. Sanders* and F.-S. Yen*
University of California, Los Angeles, University of Louisvillle, and Veterans Administration Hospital, Brentwood

9:30 a.m.  
(Ca-3) The Mutagenic Action of Aflatoxins in Microorganisms.
D. F. Callen*, G. R. Mohn* and T-M. Ong
National Institute of Environmental Health Sciences

9:45 a.m.  
(Ca-4) Environmental and Laboratory Monitoring of Biotic Indicators of Heavy Metals.
W. R. Lower, V. K. Drobney*, P. S. Rose*, and C. W. Putnam*
University of Missouri, Columbia

10:00 a.m.  
(Ca-5) The Mutagenic Activity of Ferrous Sulfate for Salmonella typhimurium.
D. Brusick, F. Gletten*, D. R. Jagannath*, U. Weekes and E. Zeiger
Litton Bionetics and Food and Drug Administration

10:15 a.m.  
(Ca-6) Mutagenicity Studies on 2,4-Dinitrotoluene.
J. R. Hodgson*, M. A. Kowalski*, J. P. Glennon*, J. C. Dacre* and C-C. Lee*
Midwest Research Institute and U. S. Army Medical Bioengineering Research and Development Laboratory

10:30 a.m.  
(Ca-7) Polybrominated Biphenyl Non Teratogenic, c-Mitosis Synergist in Rat.
G. Ficsor and G. F. Wertz
Western Michigan University
SATURDAY

(Ca) SUSPECT COMPOUNDS (continued)

10:45 a.m.  (Ca-8) Mutagenicity of Anthraquinone Derivatives and Related Compounds: *In vitro* Tests with the *Salmonella typhimurium/Microsomal System.*
J. P. Brown and R. J. Brown*
Dynapal

11:00 a.m.  (Ca-9) Mutagenic Activity of Drinking Water Concentrates.
V. F. Simmon and R. G. Tardiff
Stanford Research Institute and National Environmental Research Institute (EPA), Cincinnati

11:15 a.m.  (Ca-10) Plant Activation of Herbicides into Mutagens--The Mutagenicity of Field-Applied Atrazine on Maize Germ Cells.
M. J. Plewa and J. M. Gentile
University of Illinois and Yale University Medical School

11:30 a.m.  (Ca-11) Plant Activation of Herbicides into Mutagens--The Mutagenicity of Atrazine Metabolites in Maize Kernels.
J. M. Gentile and M. J. Plewa
Yale University Medical School and University of Illinois

11:45 a.m.  (Ca-12) Genetic Activity of Dithiocarbamate and Thio-carbamoyl Disulfide Fungicides in *Saccharomyces cerevisiae, Salmonella typhimurium* and *Escherichia coli.*
G. Warren*, P. D. Skaar* and S. J. Rogers*
Montana State University

- NOTES -
SATURDAY
South Grand Ballroom
9:00 a.m.

(Cb) SYSTEM DEVELOPMENT: MAMMALIAN

Chairman: E. R. Soares
NIHES

Co-Chairman: G. Sega
Oak Ridge National Lab.

9:00 a.m. (Cb-1) In Vitro Culture of Mouse Embryos After TEM-Treatment of the Fathers.
K. Burki* and W. Sheridan
National Institute of Environmental Health Sciences

9:15 a.m. (Cb-2) Predictability of Dominant Lethal Effects from Study of Fertility and General Reproductive Performance (Segment I).
M. S. P. Manandhar and B. A. Jackson
Lederle Laboratories

9:30 a.m. (Cb-3) Induction of Heritable Translocations with Methyl Methane-Sulfonate in Male Mice.
R. Lang and I-D. Adler
Schering AG, Research Laboratories and Gesellschaft fur Strahlen- und Umweltforschung, Germany

9:45 a.m. (Cb-4) Induction of Reciprocal Translocation in Mice by Triethylene-Melamine.
C. W. Sheu, F. M. Moreland, E. J. Oswald and S. Green
Food and Drug Administration, Washington, D. C.

10:00 a.m. (Cb-5) Studies of the Fate and Distribution of TEM in Mice.
E. R. Soares, T. Eling* and M. Anderson*
National Institute of Environmental Health Sciences

10:15 a.m. (Cb-6) Unscheduled DNA Synthesis in the Germ Cells of Male Mice Treated In Vivo with Chemical Mutagens Requiring Metabolic Activation.
R. E. Sotomayor*, G. A. Sega* and R. B. Cumming*
Oak Ridge National Laboratory

10:30 a.m. (Cb-7) Autoradiographic Studies of Unscheduled DNA Synthesis in the Germ Cells of Male Mice Treated In Vivo with MMS and X-Rays.
R. E. Sotomayor*, G. A. Sega* and R. B. Cumming*
Oak Ridge National Laboratory
10:45 a.m. (Cb-8) Comparisons Between the Unscheduled DNA Synthesis Induced by Two Alkyl Methanesulfonates and by X-Rays in the Germ Cells of Male Mice.
G. A. Sega* and R. E. Sotomayor*
Oak Ridge National Laboratory

11:00 a.m. (Cb-9) Combined Effects of Methyl Methanesulfonate and X-Rays on Unscheduled DNA Synthesis in Early Spermatids of the Mouse.
D. A. Carpenter* and G. A. Sega*
Oak Ridge National Laboratory

11:15 a.m. (Cb-10) The In Vivo Coat-Color Somatic-Mutation Method in Chemical Mutagenesis Studies in the Mouse.
L. B. Russell
Oak Ridge National Laboratory

11:30 a.m. (Cb-11) Estimation of the Genetic Hazards of Radiation in Women on the Basis of Mouse Specific-locus Mutation Data.
W. L. Russell
Oak Ridge National Laboratory

- NOTES -
SATURDAY
North Grand Ballroom
2:00 p.m.

(Da) SYMPOSIUM: CURRENT PROGRESS IN CHROMOSOMAL
ABERRATION RESEARCH

Chairman: W. M. Generoso
Oak Ridge National Laboratory

(Da-1) Induced DNA Lesions, DNA Repair, and Chromosomal
Aberration Formation.
M. A. Bender
Brookhaven National Laboratory

(Da-2) Inducibility by Chemical Mutagens of Chromosome Aberrations
in Male and Female Germ Cells of Mice.
W. M. Generoso
Oak Ridge National Laboratory

(Da-3) Are Chromosome Aberrations a Reliable Index of Genetic
Damage?
J. G. Brewen
Oak Ridge National Laboratory

- NOTES -
SUNDAY, MARCH 14, 1976
Center Grand Ballroom
10:00 a.m.

(Ea) SYSTEM DEVELOPMENT: SOMATIC CELL CULTURE
AND SISTER-STRAND EXCHANGE

Chairman: S. E. Bloom
Cornell University

Co-Chairman: D. Matheson
Litton Bionetics, Inc.

10:00 a.m.  (Ea-1) Measurement of Mutation at the HGPRT Locus in
            Diploid Human Lymphoblasts.
            W. G. Thilly
            Massachusetts Institute of Technology

10:15 a.m.  (Ea-2) Dose-Response Relationships of Cytotoxicity and
            Mutagenicity of Monofunctional Alkylating Agents in
            Chinese Hamster Ovary Cells.
            D. B. Couch* and A. W. Hsie
            The University of Tennessee and Oak Ridge National Laboratory

10:30 a.m.  (Ea-3) Liver-Homogenate Mediated Mutagenesis in Chinese
            Hamster V79 Cells by Chemical Carcinogens.
            D. F. Krahn* and C. Heidelberger
            University of Wisconsin

10:45 a.m.  (Ea-4) Use of L5178Y (TK\(^+/-\)) Mouse Lymphoma Cell Line
            Coupled with an In Vitro Microsomal Enzyme Activation
            System to Study Chemical Promutagens.
            D. Matheson* and B. Creasy*
            Litton Bionetics, Inc.

11:00 a.m.  (Ea-5) An In Vivo BrdU Technique for Analyzing the
            Induction by Mutagens of Sister Chromatid Exchanges in
            Mouse Spermatogonia.
            J. W. Allen and S. A. Latt*
            Children's Hospital Medical Center and Harvard Medical School

11:15 a.m.  (Ea-6) Detection of Sister Chromatid Exchanges in Chick
            Embryos Exposed to Bromodeoxyuridine.
            S. E. Bloom and T. C. Hsu
            Cornell University and M. D. Anderson Hospital and Tumor
            Institute

11:30 a.m.  (Ea-7) Detection of Sister Chromatid Exchange Following
            5-Bromodeoxyuridine Incorporation in the Central Mudminnow.
            A. D. Kligerman and S. E. Bloom
            Cornell University
SUNDAY

(Ea) SYSTEM DEVELOPMENT: SOMATIC CELL CULTURE AND SISTER-STRAND EXCHANGE (continued)

11:45 a.m.  (Ea-8) Sister Chromatid Exchange and Methylazoxymethanol Acetate.
L. A. Evans*, M. Kevin* and E. C. Jenkins
Fordham University and New York State Institute for Basic Research in Mental Retardation

12:00 p.m.  (Ea-9) Dominant Genetic Damage Induced in Male B6CF1 Mice by γ-Rays, Neutrons, and Alpha Particles.
D. Grahn, B. Frystak*, J. J. Russell* and A. Lindenbaum*
Argonne National Laboratory

- NOTES -
SUNDAY
South Grand Ballroom
10:00 a.m.

(Eb) SYSTEM DEVELOPMENT: PLANT AND INSECT

Chairwoman: R. C. Sparrow
Brookhaven National Lab.

Co-Chairman: A. H. Sparrow
Brookhaven National Lab.

10:00 a.m. (Eb-1) Variability in Spontaneous Somatic Mutation Frequencies of a Flower Color Locus in Tradescantia.
A. H. Sparrow and R. C. Sparrow
Brookhaven National Laboratory

10:15 a.m. (Eb-2) Response of Somatic Mutation Frequency in Tradescantia to Exposure Time and Concentration of Gaseous Mutagens.
A. H. Sparrow and L. A. Schairer
Brookhaven National Laboratory

10:30 a.m. (Eb-3) Dosimetry of Tritiated 1,2-dibromoethane in Floral Tissues of Tradescantia.
C. H. Nauman, P. J. Klotz* and A. H. Sparrow
Brookhaven National Laboratory

10:45 a.m. (Eb-4) Screening for Induced Chromosome Loss and Non-Disjunction in Drosophila melanogaster.
S. Mittler
Northern Illinois University

11:00 a.m. (Eb-5) Evaluation of the Drosophila System for Mutagenesis Screening.
R. Valencia
WARF Institute, Inc.

11:15 a.m. (Eb-6) The Effects of Dietary Deficiencies for Choline and Nicotinic Acid on the Sensitivity of Drosophila melanogaster to Mutagenic Treatment.
B. W. Geer and D. L. Reno*
Knox College

11:30 a.m. (Eb-7) Thiotepa-Induced Effects on Genetic Fitness in Aedes aegypti.
P. H. Rodriguez
The University of Texas at San Antonio
SUNDAY
North Grand Ballroom
2:00 p.m.

(Fa) SECOND ANNUAL INDUSTRIAL SYMPOSIUM:
GENETIC SAFETY EVALUATION OF INDUSTRIAL
CHEMICALS

Chairman: V. A. Ray
Pfizer Pharmaceuticals

(Fa-1) An Approach to the Problem.
D. Brusick
Litton Bionetics, Inc.

(Fa-2) Safety Evaluation of Nitrilotriacetic Acid.
L. G. Scharpf, Jr.
Monsanto Industrial Chemicals Co.

(Fa-3) The Role of CIIT.
L. Golberg
Chemical Industry Institute of Toxicology

4:00-5:00 p.m.
North Grand Ballroom

ANNUAL BUSINESS MEETING

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MONDAY, MARCH 15, 1976
North and Center Grand Ballrooms
9:00 a.m. - 12:00 p.m.

(Ga) JOINT ENVIRONMENTAL MUTAGEN SOCIETY/SOCIETY OF TOXICOLOGISTS
SYMPOSIUM: THE LOW-DOSE EXTRAPOLATION DILEMMA

Moderator: J. W. Drake
University of Illinois, Urbana

(Ga-1) The Use of Models for Low-Dose Extrapolation.
D. W. Gaylor
National Center for Toxicological Research

(Ga-2) Restraints for Consideration before Extrapolation.
C. S. Weil
Carnegie-Mellon University

- NOTES -
abstracts

(Aa-1)
"N-Hydroxy-2-aminofluorene is the Principal Mutagen Produced from 2-Acetylaminofluorene." Daniel L. Stout*, James N. Baptist*, Thomas S. Matney, and Charles R. Shaw, The University of Texas System Cancer Center and Health Science Center, Houston, Texas 77025.

N-hydroxy-2-acetylaminofluorene (N-OH-AAF) is considered to be a key intermediate in the metabolism of AAF to carcinogenic and mutagenic products. The in vitro enzymatic production of mutagenic derivatives from this intermediate was studied in a soluble enzyme system prepared from rat liver. Mutagenic metabolites generated in this system were recovered by extraction into benzene. The components in the extracts were separated by thin layer, and liquid-solid chromatography. Salmonella typhimurium TAL538 was used to determine optimum conditions for mutagen production and to test the mutagenicity of the several metabolites recovered.

We found the mutagen produced to be relatively stable in the incubation mixture, and its decay appeared to be enzymatic. No mutagenic activity remained in the incubation mixture after benzene extraction. Using the bacterial tester, TAL538, and the chromatographic procedures we have accounted for the disposition of mutagen produced, and determined that N-hydroxy-2-aminofluorene is the principal mutagen produced from N-OH-AAF in the soluble enzyme system.

(Aa-2)
"5-Halogenated Uracil Base Analog Mutagenesis." Marva F. Walton* and Robert B. Cumming*, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

5-Chlorouracil, a product of water chlorination, when given to mice in drinking water, is measurably incorporated into liver and testicular DNA. However, no specific locus or dominant lethal mutations have been detected in the exposed animals.

5-Chlorouracil, as well as 5-bromouracil and 5-iodouracil, are also incorporated into the DNA of many types of bacteria, replacing thymine. In at least some bacterial strains all three agents are highly mutagenic when incorporated into DNA. The classical interpretation of 5-halogenated base analog mutagenesis is that the mutations produced are A-T → G-C or G-C → A-T transitions resulting from mispairing of the halogenated base with guanine rather than adenine during DNA replication. This interpretation has recently been challenged and the mutagenic mechanism for these base analogs is under active discussion.

In E. coli, strain WP2, the three base analogs mentioned above are all highly mutagenic. Both 5-chlorouracil and 5-bromouracil give mutation frequencies of approximately 1000-fold the spontaneous frequency following a one-hour exposure to medium containing 50
ug/ml of base analog. At this time there is substantial replacement of the thymine in the bacterial DNA with the halogen-containing base. It has not been possible to make quantitative measurements of mutations/locus/thymine substitution/replication. This is true for several reasons, but the most important is that the frequency of expressed mutations is not a linear function of the amount of base analog incorporated into the DNA. At higher levels of incorporation than the optimum, the mutation frequency observed drops, and at very high incorporation levels the mutation frequency is no higher than spontaneous.

These results indicate either an inhibition of the production or expression of mutations at high levels of the mutagen, or a basic mechanism of base analog mutagenesis different from any so far proposed.

Research sponsored by the U. S. Energy Research and Development Administration under contract with the Union Carbide Corporation.

(Aa-3)

"Mutagenicity of Compounds Structurally Related to ICR-170 and ICR-191 in the Presence of O_2 or N_2 in Neurospora crassa." Herman E. Brockman and Wen-zong Whong*, Department of Biological Sciences, Illinois State University, Normal, Illinois 61761.

Although the acridine mustards ICR-170 and ICR-191 differ only in that ICR-170 has an ethyl group rather than a hydrogen on the amino nitrogen containing the 2-chloroethyl group, the two compounds have strikingly different mutagenicities in eucaryotes and procaryotes. Our laboratory has reported on the mutagenicities of eight ICR-compounds that contain the ICR-170-type or ICR-191-type side chain. We have continued this systematic comparison of ICR-compounds structurally related to ICR-170 and ICR-191 by studying reversion of an adenine-3 mutant of N. crassa that is a presumptive frameshift. Because an anoxia effect was observed with ICR-compounds that were studied previously (Wong, J., Mutation Res., 31(1975)306), we compared the mutagenicity of the compounds in the presence and absence of O_2. Data will be presented from which we conclude that (1) the anoxia effect is a general phenomenon with these compounds, although the degree of the effect depends on the compound; (2) the ICR-170-type analogue is generally more mutagenic than the ICR-191-type analogue for each type of heterocyclic nucleus, although the type of nucleus determines how great the difference is; and (3) among the compounds with the ICR-170-type side chain, the type of nucleus has a dramatic effect on mutagenicity. Our data will be compared to those of Creech and coworkers (Creech et al., J. Med. Chem. 15 (1972)739) on the ability of these compounds to induce
reversion in a frameshift mutant of *Salmonella typhimurium* and to possess antitumor activity against ascites tumors in mice.

Research supported by U. S. Energy Research and Development Administration under Contract No. E(11-1)-1314 with Illinois State University.

(Aa-4)

Our laboratory is attempting to identify a set of tester mutants among rII mutants of bacteriophage T4 whose reversion to wild-type or conversion to a different mutant codon will allow us to distinguish by a simple genetic test the occurrence of all possible base pair substitutions. These tester strains would have primary value in identifying which base pair changes could be induced by a mutagen and thereby suggest possible targets and mechanisms of mutagenesis.

A:T→G:C transitions may be measured by the conversion of UAA (ochre) to either UAG (amber) or UGA (opal) mutants. The reciprocal measurement of G:C→A:T transitions is complicated by the ability of ochre-suppressing *E. coli* hosts to suppress UAG mutants and the leakiness of UGA mutants of T4 on these hosts. However, some UGA mutants have now been found which can be used.


Transversions arising at G:C sites can be detected specifically if a mutagen does not induce A:T-site mutations by looking at the reversion of UAG and UGA mutants. Heat is such a mutagen. Heat mutagenesis has identified one rII mutant which reverts only by G:C-site transversion.

In an attempt to define all four transversion pathways specifically, we have searched among rII missense mutants which can be induced to convert to nonsense mutants by single-base-pair substitutions. If the original missense codon can be deduced, a specific pathway of conversion is defined. Several convertible missense mutants have been found.
Nucleotide changes occurring spontaneously and induced by a variety of mutagens previously were determined in the yeast *Saccharomyces cerevisiae* by examining the reversion rates of *cyc1* mutants that have known pathways of reversion and by examining the amino acid replacements in revertant iso-1-cytochromes c. The iso-1-cytochrome c system also can be used to determine mutagenic specificities from the distribution of types of forward *cyc1* mutants. Such investigations of forward mutants are a sensitive means of examining highly mutable sites, the so-called “hotspots” and of determining the dependence of mutations on positions within the gene.

We have previously reported the isolation and characterization of 210 *cyc1* mutants that were mainly obtained spontaneously or induced with UV or ICR-170 (Sherman *et al.*, Genetics 77, 255 [1974]). Amino acid replacement data and recombination studies with a set of deletion mutants made it possible to assign the mutational sites of the *cyc1* mutants to exact or approximate amino acid positions of iso-1-cytochrome c (Sherman *et al.*, Genetics 81, 51 [1975]). Some of the base-pair changes producing the *cyc1* mutants could be deduced from the nature of the *cyc1* lesion and from the corresponding normal amino acid residue.

Studies of mutagenic specificities with forward *cyc1* have been extended by examining 50 or more mutants induced with ethyl methanesulfonate (EMS), nitrous acid (NA), diepoxybutane (DBB) and nitroquinoline oxide (NQO) and by examining additional 60 spontaneous mutants. The characterization and mapping of these new mutants are in varying stages of completion. With the information at hand it is clear that the chemical agents ICR-170, EMS, DEB, and NA preferentially induce mutations at particular sites. The distribution and character of the mutants indicate that the action of these chemical agents is dependent not only on the nature of the affected base pairs but also on the relationship to neighboring base pairs. There are even distributions of the sites of UV-induced mutants and spontaneous mutants although their occurrences appear to be far from random. (Supported by NIH grant GM12702 and by USERDA, Report No. UR-3490-872.)

"Linearity of Induced Mutagenesis at Low UV Doses in Yeast."
Friederike Eckardt* and R. H. Haynes, Department of Biology, York University, Toronto, Canada.

We have measured the frequency (mutants per survivor) of UV-induced reversion to prototrophy (ADE* and LYS*) in both excision deficient and proficient haploid strains of yeast (*Saccharomyces cerevisiae*) over a relatively wide range of doses (1 to 1500 ergs/mm²). Double log plots of these data (corrected for spontaneous revertants) show that mutation induction is linear at low doses. A transition to higher order kinetics occurs at doses near 15 ergs/mm² in excisionless strains and near 70 ergs/mm² in repair-proficient strains. In the highest observed dose range the slopes of the induction curves attain values as high as 3.
These results suggest that, at sufficiently low doses, UV mutagenesis in yeast is a single-event Poisson process, statistically independent of cell killing, and that the commonly reported quadratic kinetics observed at moderate doses can be usefully regarded as a departure from what is basically a linear process. There are three possible reasons, not mutually exclusive, for the transition to higher order kinetics: first, for presently obscure macromolecular reasons, mutation and killing may not remain statistically independent processes as doses are increased; second, the UV-sensitivity of the auxotrophs might be greater than the sensitivity of the prototrophic revertants; and third, the effective cross-section for mutagenesis might itself increase with dose. The latter effect could arise either from an increasing frequency of error-prone repair events or from the switching-on of supplementary error-prone repair pathways by the radiation itself. (Work supported by the Deutsche Forschungsgemeinschaft and the National Research Council of Canada)


Although genetic factors ultimately determine the repair capability of cells, the maximum repair rate is also fixed by the state of the particular cell type studied. The rate at which human peripheral blood lymphocytes (HPBL) are able to excise damage is only one tenth of the rate at which HPBL cultures incubated 72 hours with the mitogen concanavalin A remove acetoxy acetyl amino fluorene (AAAF)-induced damage and only about one thirtieth of the capacity of a lymphoblastoid line derived from a Burkitt's lymphoma. The initial rate of repair after AAAF-induced damage is greater than that after treatment with methyl methanesulfonate (MMS) when human lymphoma cells are tested but the initial AAAF- and MMS-induced repair rates are indistinguishable when tested with HPBL not stimulated with mitogen. An interpretation of these observations is that agents such as AAAF require a rate-limiting excision enzyme for their repair, that this enzyme is particularly deficient in non-proliferating cells and that the enzyme is not required for the repair of MMS-induced damage.

We tested one prediction of this hypothesis using developing chick retinoblasts. As differentiation proceeds these cells lose their ability to synthesize DNA. We found that loss of DNA synthetic ability is correlated with a loss of repair capacity although the kinetics of the decline differ for DNA synthesis and repair. The decline in repair cannot be accounted for by loss of thymidine kinase or DNA polymerase.
These results imply that quantitative estimates of DNA repair require specification of the physiological and developmental state of the cells used in addition to standardization of their genetic type. Insofar as repair and replication may be uncoupled, they also suggest the existence of physiological states in which cells may be particularly susceptible to mutagenesis due to a deficiency in repair.

(This work was supported by grants from ERDA E(11-1)2040 and the National Institutes of Health (GM 07816, CA 14599-03).

(Aa-8)

"Interspecific cytogenetics: the quantitative effect of interphase chromosome disposition." Peter Clifford*, University of California, Berkeley, California, 94720.

A number of recent and proposed studies are concerned with interspecific comparisons of mammalian radiosensitivity using the irradiated lymphocyte system. Taking as the endpoint the total centric yield at the first mitosis following irradiation Brewen et al. have proposed a linear interpolatory formula for the relationship between the centric yield and the number of arms in the specific karyotype. The model of Savage and Papworth provides a structural basis for a relationship which although nonlinear is in broad agreement with observation.

A temptation exists to attribute deviations from the theoretical predictions to properties of the karyotype which have been ignored—specifically the relative arm lengths. The purpose of this paper is to show that under a variety of hypotheses about the disposition of the interphase chromosomes the effect of differences in relative arm lengths is minor and thus cannot be relied upon to explain exceptions to Brewen's rule.

This investigation was supported in part by the auspices of the U.S. Energy Research and Development Agency and in part by the Gesellschaft fur Strahlen- und Umweltforschung, Neuherberg bei Munchen.

(Aa-9)

"Some Useful Equations for Determining Sample Size in Spontaneous and Induced Mutation Experiments Where the Standard Deviation is Proportional to the Mean," K. H. Thompson* and Rhoda C. Sparrow*, Biology Department, Brookhaven National Laboratory, Upton, New York 11973.
In mutagenesis experiments with *Tradescantia* it is frequently desirable to estimate the number of mutant events (E) or the number of flowers (n) or of stamen hairs that must be scored such that the standard error (s) of the mean number of mutant events per hair (X = E/nh, where h is the number of hairs per flower) is a predetermined proportion or ratio (r = s/X) of the mean. Using *Tradescantia* exposed to 0-500 rads it has been observed that the logarithm of the standard deviation (s) is linearly proportional to the logarithm of the number of mutant events per hair (X).

\[
\log(s) = -1.2 + 0.5 \log(X)
\]

This relationship is used to show that the number of flowers required to produce a fixed ratio of standard error to mean may be estimated as

\[
\log(n) = -2.4 - 2 \log(r) - \log(X).
\]

It may be further shown that the total number of mutant events (E) required to produce a predetermined ratio may be estimated as

\[
\log(E) = -2.4 - 2 \log(r) + \log(h).
\]

This value of E depends only on a fixed value of r and an independent estimate of h and is independent of dose and the number of expected events per hair. Thus, a predictable degree of statistical validity can be obtained if scoring is continued until a specified number of mutant events (E) has been observed. This constant relationship has proven extremely useful in *Tradescantia* mutation studies and should be of general utility in designing experiments involving the counting of spontaneous or induced mutations in other organisms.

Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Energy Research and Development Administration.

(Aa-10)

"Reversibility of the Inhibitory Effects of Hexachlorophene and Decanoic Acid in Bacillus subtilis." Barbara Chernov Levin*, and Ernst Freese, NIH, Bethesda, Maryland 20014.

Growth, respiration, ATP synthesis and amino acid uptake are completely inhibited by the minimal inhibitory amount of 100 p moles of hexachlorophene added per ml of a *Bacillus subtilis* culture, grown in a 1% casein hydrolysate medium to an O.D.600 of approximately 0.3. At a higher cell density, more hexachlorophene is needed to produce the same inhibition. In contrast, decanoic acid is needed at a much higher concentration (1 μ mole/ml) to inhibit completely the same metabolic functions and its inhibitory strength does not depend on the cell density. Furthermore, washing the cells with growth medium reverses the inhibition of decanoate but not that of hexachlorophene. However, addition of bovine serum albumin (1%) reverses the inhibition by either compound apparently by binding the lipophilic acids and thus reducing their concentration on the cells and in the medium. The inhibitory effects of hexachlorophene are also reversed by the addition of untreated cells to the inhibited culture. The differences are attributed to the much higher oil/water distribution coefficient of hexachlorophene as compared to decanoate. At their respective minimal inhibitory concentrations, more than 80% of the hexachlorophene but less than 1% of the decanoic acid are attached to the cells.
The addition of glucose (0.5%) or malate (10 mM) allows the resumption of growth in the presence of the minimal inhibitory concentration of either inhibitor, i.e., the transport of glucose and malate is not inhibited under these conditions. Since both hexachlorophene and decanoic acid apparently destroy the proton gradient needed for amino acid uptake, glucose and malate must be able to enter the cells by mechanisms other than the proton gradient.

(Aa-11)
"Effect of Excision Repair on Azide Induced Mutagenesis."
Andris Kleinhofs*, Jane A. Smith*, and Robert A. Milan,
Washington State University, Pullman, Washington 99163.

Azide mutagenesis was investigated in Salmonella typhimurium and Escherichia coli. Metabolic inhibitors, similar in action to azide, did not induce mutations in S. typhimurium strain TA1530 thus ruling out the possibility that azide mutagenesis was due to peroxide accumulation. Azide was highly effective in inducing his revertants in uvrB derivatives of S. typhimurium his G46 and in inducing high frequencies of 5-fluorouracil resistant mutants in uvrA derivatives of E. coli B/r WP2. In uvr+ derivatives of these strains, azide was not effective or only a marginal mutagen, demonstrating that the bacterial excision-repair system could repair azide-induced damage. The presence of recB or recC genes in combination with uvrA increased E. coli sensitivity to azide killing, but depressed azide mutagenicity. These results are similar to those reported for U.V. induced mutagenesis with the E. coli strains and point to post-replication repair as the error-prone step in the repair process. Azide mutagenesis specificity is, however, unique and different from U.V. as demonstrated by the inability of azide to revert the ochre try locus in E. coli WP29. These results suggest that the initial DNA damage induced by azide is different from U.V. induced DNA damage. (Supported by NIH Grant ES01009.)

(AB-1)
"Use of the Spot Test for the Detection of Mutagenic Activities of Environmental Agents in Neurospora crassa." Tong-man Ong, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709.

The adenine-3 (ad-3) forward mutation test system of Neurospora crassa, developed by F. J. de Serres and his co-workers, has been shown to be one of the best systems for studying the mutagenicity and mutagenic specificity of chemical agents. With this test system, recessive lethal mutations resulting from either chromosome deletions or point mutations can be recovered at the ad-3 region. The isolated mutants can
then be characterized by a series of genetic tests and the
presumptive type of genetic alterations at the molecular level
can be determined. The limitations of this test system, however,
are that one cannot screen for the mutagenicity of a large number
of chemicals in a short period of time and that not every
laboratory can use this test system.

Hundreds of ad-3 mutants of N. crassa induced by a variety of
chemicals were tested for the reversibility with mutagens. Three
highly reversible mutants (2 presumptive base-pair substitution
mutants and 1 presumptive frameshift mutant) have been selected
and a spot test system with these 3 testers has been developed.
The mutagenic activities of 42 chemical carcinogens and/or
environmental agents have been tested with this test system. The
results, which are comparable to the results obtained from
forward mutation experiments, will be discussed.

The development of the spot test in N. crassa has made the
ad-3 test system a more useful system for the detection and
evaluation of mutagenic activity in environmental agents. In
an attempt to increase the sensitivity to mutagenic agents, the
introduction of a UV-sensitive marker into these tester strains
is now in progress.

(Ab-2)
"Inactivation and Mutation Induction by ICR-170 in UV-
sensitive Strains of Neurospora crassa." H. Inoue*, T. Ong and
F. J. de Serres, National Institute of Environmental Health
Sciences, P. O. Box 12233, Research Triangle Park, North Carolina
27709.

A comparison of the genetic effects of ICR-170 on wild type
and five different UV-sensitive strains (upr-1, uvs-2, uvs-3
uvs-5 and uvs-6) of Neurospora crassa have been made for both
inactivation of conidia and mutation induction at the ad-3A and
ad-3B loci. All strains are essentially isogenic and carry the
genetic markers al-2, cot and pan-2. Conidia from each strain
were treated with varying concentrations of ICR-170. Treated
and untreated conidia were assayed for survival and for frequency
of ad-3 mutations by the direct method of de Serres and Kölmark
(Nature 182:1249, 1958). Results of the survival tests show that
all UV-sensitive strains, except upr-1, are approximately 3 times
more sensitive to ICR-170 than wild type. Although upr-1 is the
least sensitive to ICR-170 among the UV-sensitive strains tested,
it is more sensitive to this compound than wild type. Results of
mutation induction experiments indicate that upr-1, uvs-6 and
wild type strains have a similar mutagenic response to ICR-170,
while uvs-2 and uvs-5 are both 3 times and uvs-3 is 5 times less
mutable by ICR-170 than the wild type strain. It appears, there-
fore, that the relative mutagenic responses of the UV-sensitive
strains and the wild type to ICR-170 are different from the
relative responses observed with UV, γ-rays and MNNG. Whether
ICR-170 causes different spectra of genetic alterations is under
investigation.
Comparative studies of six compounds in *Saccharomyces cerevisiae* and *Salmonella typhimurium* reversion systems*. Majdi M. Shahin and R.C. von Borstel, Department of Genetics, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

Several procedures are available for the use of the bacterium *Salmonella typhimurium* and the yeast *Saccharomyces cerevisiae* strains for the detection of chemical mutagens. The principle of the treatment condition routinely used in *Salmonella typhimurium* is to allow the cells to divide on solid media during the action of the chemical under testing; only the revertants will be able to form visible colonies. In *S. cerevisiae*, the routinely used procedure is to treat the cells in liquid non-nutrient medium under non-growing conditions.

We compared both procedures (microsomal system for metabolic conversion was not used in these tests) using six compounds: two nitrofuran derivaties, AF-2 and SQ 18,506; three hair dye components, 1,2-diamino-4-nitrobenzene, 1,4-diaminoanthraquinone and methyl violet, as well as ethyl methanesulfonate. Of the six compounds tested in *S. cerevisiae* strain XVI85-14C using the technique routinely employed in the bacterium *Salmonella typhimurium*, only ethyl methanesulfonate was mutagen. The two nitrofuran derivatives AF-2 and SQ18,506 induced mutation in *S. cerevisiae* only when *S. cerevisiae* procedure was employed. Under both experimental conditions used, none of the three hair dyes tested was mutagenic in *S. cerevisiae*. However, the results obtained with *Salmonella typhimurium*, indicate that the hair dye 1,2, diamino-4-nitrobenzene is a mutagen. Among the strains tested, the frameshift mutants TA1538 and TA98 responded with the highest frequency of reversions, followed by the frameshift mutant TA1537. The base pair substitution mutant TA1535 did not respond to any of the three hair dyes tested. On the basis of these data, we can conclude that the procedure of *Salmonella typhimurium* is not applicable for our *S. cerevisiae* strain XVI85-14C. Research associated with the Hydrocarbon Research Center at the University of Alberta.

Detection of Mutagen-Induced Genetic Duplications in *Salmonella typhimurium.* George R. Hoffmann and Robin W. Morgan*, Meredith College, Raleigh, North Carolina 27611.

A genetic duplication that includes approximately 30 percent of the chromosome may be detected in the bacterium *Salmonella typhimurium* by selection for fast growth on medium in which L-malate is the sole carbon source. In addition to the formation of large colonies on minimal malate medium, strains containing the duplication are characterized by rough colony morphology and genetic instability of the duplication-phenotype.

Since the duplication represents a genetic alteration quite different from those commonly studied in bacteria, we are investigating the effect
of chemical mutagens and radiation on the frequency of its occurrence. Under defined conditions, the spontaneous duplication frequency is approximately $10^{-4}$ per viable cell plated. Following mutagenic treatment, the frequency is considerably increased. In addition to inducing duplications, ultraviolet light stimulates loss of the duplication from merodiploid strains. Induction of both duplication formation and loss by ultraviolet light may be explained by a recombinogenic effect of mutagens in bacteria.

(Ab-5)


A number of heterocyclic N-nitrosoamines (the series from N-nitrosoguanidine through N-nitrosododecamethylenimine, N-nitroso-piperazine and its derivatives, and N-nitrosomorpholine and its derivatives) were evaluated for mutagenicity to Salmonella typhimurium TA-1535, which responds to mutagens inducing base-pair substitutions. Both suspension and plate tests were used, with mouse and rat liver in vitro metabolic activation systems.

The mutagenic responses obtained using mouse liver were equal to, or greater than, the responses obtained using rat liver, in both the suspension and plate tests. Although it is difficult to make quantitative comparisons between plate and suspension tests, in this study both systems appeared to be responsive to the same dose ranges for the individual nitrosoamines. There was a qualitative correlation of mutagenicity with carcinogenicity; however, the relative mutagenic activities did not parallel the relative carcinogenic activities.

(Ab-6)

"Biotransformation Capacity of Extracts from Rabbit Testis-Tissue Using Microorganisms as Indicators." Peter Maier* and Heinrich V. Malling, National Institute of Environmental Health Sciences, P. O. Box 12233 Research Triangle Park, North Carolina 27709.

Because of the known interaction of nitrofurans with testicular tissue and the poor knowledge about the reductive enzyme system in mammals, AF-2 was chosen as a test compound. The chemical shows a mutagenic response per se in E. coli, due to the efficient nitro reductase systems in microorganisms. Using the multi-purpose strain E. coli 343/113 and the S-9 testis fraction of New Zealand rabbits in liquid tests, we found different mutation patterns with and without homogenate. The arg+ system shows a very sensitive dose response with the homogenate. The modifying effect of the incubation atmosphere
and the cofactors on the mutation rates were investigated. Additional data about the biotransformation capacity of microsomes and the S-150 fraction of testis are presented. The activation capacity of testis enzyme systems for nitro compounds not influenced by the microorganism metabolism is discussed.

(Ab-7)

In previous studies, the use of trimethylphosphate (TMP) as a positive control agent in the mouse dominant lethal, cytogenetic and host-mediated assays was reported. TMP was shown to be a weak mutagen in the mouse dominant lethal and bone marrow cytogenetic assays and negative in the host-mediated assay.

Additional experiments have been conducted to continue the mutagenic profile of TMP and assess its use as a positive control agent.

Salmonella/microsome test: Salmonella typhimurium, base-pair strains, GS-46, TA-92 and TA-100 and frameshift strain TA-98 were cultured with TMP (125 mg/plate) and mouse, rat or monkey liver microsomes. Regardless of the species, TMP caused the greatest number of revertants in strains TA-100 and TA-92. In contrast, few revertants appeared in strain GS-46 and virtually none in strain TA-98.

Salmonella/urine assay: Urine collected from mice which received 1750 mg/kg of TMP was added to cultures of Salmonella typhimurium strains GS-46, TA-92, TA-98 and TA-100. The number of revertants in strains TA-98 and TA-100 appeared to be slightly in excess of the control values while no revertants appeared in strains GS-46 and TA-92.

Micronucleus test: Mice were treated with distilled water, 1250, 1500 or 1750 mg/kg of TMP and sacrificed at 6, 24 and 48 hours. The percentage of micronuclei observed showed a time-related and dose-related increase which agreed with the amount of chromosome damage seen in previous experiments.

(Ab-8)
"A Modified Host-Mediated Assay Using Human Cells" C. C. Huang, Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, N. Y. 14263.

We have developed a modified host-mediated assay system by using cultured human lymphoid cells as target cells. Induction of chromosome aberrations will be used
as an indicator for possible carcinogenicity or mutagenicity of a given compound.

Cells from several human lymphoid lines derived from tissues of cancer patients grew well, forming ascites or solid tumors after injection of $10^7$ cells into sublethally irradiated DBA/2 mice. The recultured cells from the induced tumor tissue proved to be of human origin. However, no growth was observed after direct injection of cells from normal human lymphoid lines. Recently, we have successfully grown several human lymphoid cell lines derived from both normal and cancer patients, in diffusion chambers (DC) implanted into C3H mice. The initial cell concentration per DC (Vol. $325 \pm 10 \text{ mm}^3$) was about $0.5 \times 10^6$ cells/DC was reached 4 to 5 days after implantation. Subsequently, the viable cell counts declined gradually and there were only a few viable cells after 2 weeks. The cell viability and mitotic indices of cell populations in DC during the first 3 days in hosts were found to be better than those cells grown in flasks. Effects on chromosomes of human cells in DC as well as bone marrow cells of the hosts after injection of a clastogenic agent such as trimethylphosphate are in progress.

Supported in part by grant ES-01290 from NIEHS and EPA.

(AB-9)

The development of methodology for estimating the mutagenic potential of chemicals with a priority on development of an in vivo mammalian system which will be useful for the study of induced microlesions ("point mutation", small deletions) is an important aspect of mutagenesis research. Toward this goal the techniques employed in the study of inborn errors of metabolism, with special emphasis directed toward techniques which are amenable to detection of mutations in the heterozygous condition, are being developed for utilization as methods for detecting induced microlesions in experimental animals.

Three experimental approaches to detection of metabolic errors and altered gene products are being developed. (1) Measurements of normal enzyme activity, as well as the heat stability of the enzyme protein are conducted on various enzymes obtained from liver, brain, kidney, heart and/or erythrocytes. (2) Electrophoretic studies of enzyme and non-
enzyme proteins are conducted to detect structural alterations which are not detected by changes in activity parameters. (3) The metabolite status of plasma and urine are monitored as an indication of storage diseases, metabolic blocks and other pathway alterations.

Tissue samples obtained from progeny of C57BL/6J males exposed to 600 or 300 R of irradiation are currently being examined for induced mutations as follows: thirty-five tissue enzymes are being examined for level of activity; fifteen enzymes are being assayed for heat stability; twenty proteins are being monitored for electrophoretic characteristics. Additionally, the concentration of fifty urinary and/or plasma metabolites associated with seventy known human metabolic errors is being assessed.

The utility of this approach to monitoring genetic alterations will be discussed.

(Ab-10)

The evaluation of human safety from genetic hazards of environmental chemicals is a process which encompasses a wide range of technological developments, innovative approaches, socio-economic problems and diversified professional expertise. It is a process of great dimensions, since, ideally, all chemicals in the human environment should be evaluated for potential mutagenicity. It involves the generation of mutagenicity data, progressive integration and interpretation of such data for the purpose of evaluation of possible genetic risks to man. Thus, it is a process of enormous complexity which presents itself with many problems. These include (i) selection and priority ranking of chemicals for mutagenicity testing, (ii) choice of appropriate tests, (iii) involvement of industry and labour organizations in mutagenicity testing, (iv) apparent lack of awareness that environmental chemicals are detrimental to human heredity, (v) extrapolation of experimental data to man and, (vi) evaluation of net genetic risk. In addition, there are certain misconceptions which hamper progress in this area e.g. it is still believed by many that mutagenicity tests have not yet attained that level of perfection which is a prerequisite for their incorporation into standard toxicological protocols. An attempt will be made to discuss some of these problems.
(Ca-1)
"Cytogenetic Studies of Opiate Addicts: Standard, G Banded and Sister Chromatid Exchange Analyses." Felicia Hollingsworth*, David A. Shafer*, and Arthur Falek, Georgia Mental Health Institute and Emory University, Atlanta, Georgia 30306.

Our previous cytogenetic studies from 72 hour peripheral blood leukocyte cultures have demonstrated that opiate addicts as compared with controls have significantly increased frequencies of chromosome aberrations and cells with chromosome alterations prior to and up to 30 weeks on methadone maintenance. After a year, however, there is a marked decrease of cells with chromosome damage, both for those who have and those who have not remained in the methadone program.

The present investigation will compare the frequencies of chromosome damage in the leukocytes of a series of opiate addicts prior to methadone treatment and sex and age matched controls based on standard Giemsa stained chromosomes and those obtained by G banding. Sister chromatid exchange studies will also be reported to evaluate one aspect of chromosome repair. Our findings, thus far, reaffirm the increased frequency of chromosome damage from opiate users in comparison with controls with both the standard and G banded chromosome preparations. Initial evaluations, however, indicate no consistency with regard to the sites of the chromosome breaks. The frequencies of sister chromatid exchanges in unstressed leukocyte cultures from addicts and controls treated with 10ug./ml. BuDR and grown in the dark for the 72 hour culture period are not significantly different from one another. The results of current studies of comparative sister chromatid exchange frequencies after the cultures are initially stressed with U.V. as well as detailed information on the other aspects of this study will be reported.

(Ca-2)
"Cytogenetic Effects of In Vivo Administration of Methadone and L-Alpha-Acetyl Methadol (LAAM)." Steven S. Matsuyama, V. Charuvastra*, Lissy F. Jarvik, Tsu-ker Fu, Kayellen Sanders**, and Fu-sun Yen†. Department of Psychiatry, University of California, Los Angeles, †Department of Pediatrics, University of Louisville, and Veterans Administration Hospital (Brentwood), Los Angeles, California 90073.

Methadone maintenance for the treatment of heroin addicts is widely accepted. In addition, l-alpha-acetyl methadol (LAAM), an analogue of methadone, is being clinically tested because of its longer duration of action requiring only three doses per week rather than the daily regimen required for methadone. Aside from the comparative clinical efficacy of these two drugs, there is the question of chromosome damage resulting from their ingestion, and the present pilot study was carried out to investigate the occurrence of such damage. Chromosomes of peripheral lymphocytes were examined in 28 subjects participating in a VA-SAODAP cooperative study of LAAM versus methadone after nearly 40 weeks
of maintenance therapy while still on active medication. Of the 28 drug users, 11 were maintained on LAAM at 80 mg three times per week, while the remaining 17 were on methadone maintenance, 8 at 50 mg/day and 9 at 100 mg/day. For comparison, 10 non-drug users were also included in the study. The mean chromosome break frequencies were similar in all four groups.

(Ca-3)

The mutagenicity of aflatoxin B₁ and G₁ were studied in E. coli and Saccharomyces cerevisiae. The test systems used were arg reversion in the E. coli strain K-12/343/113 and intragenic conversion at the ade-2 and try-5 loci in the yeast strain D4. Aflatoxin B₁ and G₁ did not exhibit any mutagenic properties in these two systems unless liver homogenates prepared from phenobarbital treated hamster and a NADPH generating system were added to the treatment solution. Under these conditions aflatoxin B₁, at a concentration of 83 µg/ml, increased the arg revertants from 5.4/10⁶ survivors to 142.6/10⁶ survivors in E. coli and at a concentration of 100 µg/ml the ade convertant frequency increased from 3.3/10⁶ survivors to 93.0/10⁶ survivors in yeast. Aflatoxin G₁ was less mutagenic than B₁ since a concentration of 83 µg/ml increased the arg revertants from 21.9/10⁶ survivors to 70.7/10⁶ survivors in E. coli and had no significant effect in yeast. These results parallel the relative mutagenic response in Neurospora crassa and the relative carcinogenicity of these two aflatoxins.

It has been suggested that the active metabolite of aflatoxin B₁ is an epoxide which can be converted to a dihydrodiol by an epoxide hydrase. This suggestion is supported by the observed increase in the frequency of ade-3 mutations in N. crassa and ade-2 or try-5 conversions in yeast induced by aflatoxin B₁ in the presence of the epoxy hydrase inhibitor, 1,2-Epoxy-3,3',3'-trichloro-propane, in the test systems. This inhibitor was itself highly mutagenic but this mutagenic activity was eliminated in the presence of liver homogenate.

(Ca-4)
"Environmental and Laboratory Monitoring of Biotic Indicators of Heavy Metals." William R. Lower, V. Kay Drobney*, Philip S. Rose* and Charles W. Putnam*, Environmental Trace Substances Research Center, University of Missouri, Columbia, Missouri 65201.

Previous studies from this laboratory have established the use of Drosophila as environmental indicators of lead, cadmium,
copper and zinc. These studies were carried out in the environs of a lead smelting operation in Southeastern Missouri. Subsequently, using isoenzyme loci, we have found significant gene frequency differences in collections of D. melanogaster from the smelter area, with differences correlated to metal concentration and distance from the smelter. Of eighteen loci commonly monitored, half show changes in allelic frequency. Seasonal effects are also found to be influenced by concentration of metals. Triose phosphate isomerase, esterase-c, esterase-6, alcohol dehydrogenase, and phosphoglucomutase showed particular sensitivity to lead or cadmium.

In addition, Drosophila populations were maintained in a controlled laboratory environment on media enriched with 100 and 500 parts per million lead, 10 and 50 ppm cadmium, 170 ppm sodium chloride and one control media.

Fifty individuals from each population were analyzed for 18 enzymes and allelic frequencies compared. Esterase-6 and triose phosphate isomerase showed the most consistent sensitivity to lead enrichment. Cadmium enrichment was best reflected by alcohol dehydrogenase, malic dehydrogenase, malic dehydrogenase-2, triose phosphate isomerase and esterase-6.

As a further assay, Peromyscus were caught at several of the Drosophila collecting stations. Preliminary tests for urinary aminolevulinic acid and blood protoporphyrin were performed along with routine analyses of several organs and blood for concentrations of lead, cadmium, copper and zinc. Values from these tests indicate a possible correlation between both proximity to heavy metal source and reduction to background concentrations after termination of exposure. Possible relationships between gene frequencies and ALA are being investigated.

(Ca-5)

Ferrous sulfate, a common dietary supplement and food additive, was found to be mutagenic for Salmonella typhimurium. The compound induced reverse mutations in TA-1537 and TA-1538, but not in TA-1535. The mutagenic activity of ferrous sulfate was most pronounced in suspension assays coupled with an in vitro microsomal enzyme activation system. Liver microsomal enzyme preparations from mouse, rat, guinea pig, monkey, and human have been used. Variable and weak mutagenic activity has been obtained in non-
activation suspension tests. Several approaches have been made to elucidate the mechanism of this chemical. Selection and free radical stabilization have been tentatively ruled out at the present time. Data and possible mutagenic mechanisms of this compound will be presented. A portion of this work was supported by a contract with the Food and Drug Administration (Contract Number FDA 223-74-2104).

(Ca-6)
"Mutagenicity Studies on 2,4-Dinitrotoluene." John R. Hodgson*, Mary A. Kowalski*, John P. Glennon*, Jack C. Dacre* and Cheng-Chun Lee; Pharmacology and Toxicology Section, Midwest Research Institute, Kansas City, Missouri, 64110 and U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Maryland, 21701.

As part of an extensive safety evaluation program of munition compounds, we have studied the mutagenic potential of 2,4-dinitrotoluene (2,4-DNT). The mutagenic effect of 2,4-DNT on germinal cells was studied using the dominant lethal assay on rats fed a diet containing 2,4-DNT for 13 weeks. Females mated with males treated with 0.2% 2,4-DNT showed a significant increase in the number of dead implants/total implants over control animals. Somatic cell mutation effects were studied by cytogenetic analysis of lymphocyte and kidney cultures derived from rats fed 0.2% of 2,4-DNT for 19 weeks. No increase in the frequency of translocations or chromatid breaks were observed in either the lymphocyte or kidney cultures. Significant increases in the frequency of chromatid gaps were observed, however, in kidney cultures after 5 weeks and in lymphocytes at 19 weeks. This would suggest that 2,4-DNT has a potential for inducing damage in somatic cells. In vitro studies using the CHO-K1 test system was negative. On the other hand, microbial tests using the TA 1535 series of histidine mutant Salmonella typhimurium strains indicated that 2,4-DNT is capable of producing base-pair mutations. The results of these studies indicate the importance of further testing of 2,4-DNT to ascertain its potential risk to human health. [Research supported by U.S. Army Medical Research and Development Command, Washington, D.C., 20314 under Contract No. DAMD-17-74-C-4073.]

(Ca-7)
"Polybrominated Biphenyl Non Teratogenic, c-Mitosis Synergist in Rat". Gyula Picsor and Gary F. Wertz, Department of Biology, Western Michigan University, 49008.

In 1973 the fire retardant chemical polybrominated biphenyl (PBB) was accidentally mixed with livestock feed in the State of Michigan.
As a result of the feed mixup in 1974 alone 11,000 PBB-contaminated beef and dairy cattle, 1,900 hogs, 1.5 million chickens and 4.4 million eggs had to be destroyed. Contaminated cattle had PBB blood levels of up to several thousand ppm. Several hundred farm families who consumed their own PBB-contaminated livestock products were also contaminated with PBB. In contaminated humans PBB levels were about a thousand fold below livestock levels. While many cattle died due to PBB-induced ailments and some pregnant animals may have aborted, there is no clear-cut evidence for similar effects in humans to date. Nevertheless great concern still remains about the potential health effects of PBB which prompted us to investigate its possible teratogenic and c-mitotic effects.

For the teratogenic study 15 pregnant rats were force fed with 1 ml corn oil containing 100 μg PBB/g b.w. six times in two day intervals beginning on the 6th day of pregnancy. 14 control animals received only corn oil. On the 19th day of pregnancy the animals were killed and 376 fetuses were weighed, sexed, visually observed for cleft palates, missing digits and gross malformations and were stained with Alizarin Red S for skeletal abnormalities. Dead implants were also counted. For all parameters no difference was found between control and treated groups. There was also no difference in the weight gain and general health of treated and control animals.

For cytogenetic analysis half of 10 control and of 12 PBB-treated animals were treated with 5 μg colchicine/g b.w., 5 h before killing on the 19th day of pregnancy. After killing the bone marrow of these animals was processed for mitotic figures. Mitotic index, metaphase index, the frequency of polyploid metaphases and the number of chromosomes per metaphase spread were determined. Animals treated with both PBB and colchicine had higher metaphase and mitotic indices than non-treated or singly treated animals. Colchicine alone or in combination with PBB decreased the frequency of polyploid metaphases compared to non-treated or PBB-treated animals. Upon casual observation of over a thousand metaphase spreads, chromosome aberrations were not evident.

Stimulating discussions with Dr. N. S. Hayner of the Michigan Department of Public Health and with Dr. H. C. Zindel and coworkers of the Department of Poultry Science of Michigan State University are gratefully acknowledged. Part of this research was supported by grants to G. F. W. from the Graduate College and to Debra Liston Paden from the Honors College of Western Michigan University.

(Ca-8)

Naturally occurring and synthetic anthraquinones have been employed as coloring agents in foods, drugs, cosmetics, hair dyes and textiles. Various plant anthraquinone-glycosides traditionally have found use in purgative preparations (rhubarb, senna, cascara sagrada)
and certain aglycones (e.g., 1,8 dihydroxy anthraquinone) are marketed as laxative agents.

Ninety 9,10-anthraquinone (AQ) derivatives and related anthracene derivatives were screened for mutagenicity with five Salmonella typhimurium tester strains and mammalian microsomal activation. About 35% of the compounds tested are considered to be mutagenic. Three patterns of mutagenesis were apparent: (1) Direct frameshift mutagenesis by certain AQ compounds bearing free hydroxyl groups. The most potent were anthragallol (1, 2,3-trihydroxy-AQ) purpurin (1,2,4-trihydroxy-AQ) and anthrarufin (1,5-dihydroxy-AQ). A few compounds exhibited activation by mammalian microsomal preparations and the majority of mutagenic hydroxy-AQs appeared to revert strain TA1537 (his 3076) specifically. In several instances mutagenesis was substantially reduced in the presence of an antioxidant (butylated hydroxy toluene). (2) Frameshift mutagenesis by certain AQ compounds with primary amino and in a few cases with secondary amino groups. Mammalian microsomes invariably potentiated frameshift mutagenesis, and activity with strain TA100 (sensitive to base pair substitutions) is seen in a few cases e.g., 1,2 diamino-AQ. (3) AQ compounds with one or more nitro groups. These derivatives exhibited the least specificity with regard to tester strain reverted and requirements for microsomal activation. All seven nitro AQs tested were mutagenic.

In those compounds with mixed 'mutagenic' functional groups, the type of mutagenesis observed was usually NO\textsubscript{2}>OH>NH\textsubscript{2}. AQ compounds bearing halogens, sulfonate or alkyl groups were apparently nonmutagenic, as were AQs substituted solely with secondary amines.

(Ca-9)
"Mutagenic Activity of Drinking Water Concentrates."
Vincent F. Simmon and Robert G. Tardiff, Stanford Research Institute, Menlo Park, California 94025, and National Environmental Research Institute, Environmental Protection Agency, Water Supply Research Laboratory, Cincinnati, Ohio 45268.

Two 400 gallon samples of Cincinnati tap water were each concentrated to 40 gallons by dialysis using a cellulose acetate membrane followed by a nylon membrane. The concentrates were lyophilized to powders which were then extracted with methylene chloride, acidified methylene chloride, and pentane. The solvent extracts of each sample were combine and evaporated to a volume of 10 ml under dry nitrogen. Ten per cent of the material of each sample was retained for chemical analysis and the remaining concentrate was subjected to analysis for mutagenic activity.
Each of the two concentrates was mutagenic in spot tests on Salmonella typhimurium TA100 in the presence and absence of an Aroclor 1254-stimulated, rat liver homogenate metabolic activation system. Each sample was extracted at 37°C with petroleum ether, diethyl ether, and acetone. After the diethyl ether extraction, the residual material was completely soluble in acetone. Each fraction was evaporated under nitrogen and then resuspended in dimethyl sulfoxide for mutagenic assays. Most of the organic material and mutagenic activity was in the diethyl ether and the acetone fractions. S. typhimurium strains TA100 and TA98 were reverted to histidine independence by the concentrates. The mutagenic activity was not enhanced by metabolic activation. Assays also were conducted in Saccharomyces cerevisiae D3 to determine if mitotic recombination was affected by the various fractions. A significant increase in mitotic recombination was observed in the diethyl ether fraction of one of the concentrates.

Samples of organic chemicals known to be present in drinking water in the United States have been assayed for mutagenic activity with S. typhimurium TA100. Of 130 chemicals tested to date, 15 have been found mutagenic.

(Ca-10)

We have developed a field bioassay in which reversion at the waxy locus in Zea mays microgametophytes (pollen grains) is scored. A field isolation plot was constructed and planted with maize inbreds W22 and W23. The kernels were homozygous for the waxy (wx-C) allele. The plot was divided and each sub-plot sprayed with a known concentration of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). Control sub-plots for each inbred were sprayed with water only. The plants were grown to anthesis and the tassels were removed and stored in 70% ethanol. Anthers were removed from unopened florets and the reversion frequency of wx-C to Wx-C was determined. The data indicate that there is an increase of over 10X compared to controls in the frequency of Wx-C revertant pollen grains from plants exposed to concentrations of atrazine commonly used in agriculture. The data suggest that a mutagen (s) exists in maturing plants exposed to field concentrations of atrazine that can induce genetic alterations in the germ cells of a higher eukaryote.

(Ca-11)
"Plant Activation of Herbicides into Mutagens—The Mutagenicity of Atrazine Metabolites in Maize Kernels." James M. Gentile and Michael J. Plewa, Yale University Medical School, New Haven, Connecticut 06510, and University of Illinois, Urbana, Illinois 61801.

We have previously shown that extracts from maize seedlings treated with atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino]-
s-triazine) contain an agent(s) which can induce mitotic gene conversion in *Saccharomyces cerevisiae* strain D4 (Plewa and Gentile, Maize Genet. Newsletter, 1975. 40:43; Gentile and Plewa, Mutation Res., 1975. 31:317). Since the possibility exists that a plant-mediated mutagenic agent(s) of atrazine may be passed along the food chain, we have examined extracts of kernels from atrazine-treated plants for mutagenic activity. A maize isolation plot was constructed in an untreated field. Maize inbreds W22 and W23 were planted into 16 sub-plots, and each sub-plot was treated with a known concentration of atrazine. Control sub-plots for each inbred were sprayed with water only. Sibling crosses of all plants were performed and kernels harvested. Kernels were homogenized in water and 15 fractions were prepared. All homogenates were then tested for mutagenicity by measuring their ability to induce mitotic gene conversion in *S. cerevisiae* strain D4. Extracts of kernels taken from plants treated with 5 and 20 ppm of atrazine were able to increase the rate of mitotic gene conversion approximately 2.5 and 4 X, respectively over control values, whereas a 100 ppm solution of atrazine did not increase the mitotic gene conversion rate above spontaneous levels. However, extracts taken from leaf samples of plants treated with concentrations of 5 and 25 ppm of atrazine increased the rate of mitotic gene conversion approximately 18 and 30 X, respectively over control. These data suggest that maize plants can metabolize atrazine into a mutagenic agent(s) which, although found to some degree in the kernels of the plant, is preferentially located in leaf tissues.

(Ca-12)

"Genetic Activity of Dithiocarbamate and Thiocarbamoyl Disulfide Fungicides in *Saccharomyces cerevisiae*, *Salmonella typhimurium* and *Escherichia coli*." Guyllyn Warren*, Palmer D. Skaar* and Samuel J. Rogers*, Montana State University, Bozeman, Mt. 59715.

A series of dithiocarbamate and thiocarbamoyl disulfide fungicides were compared for genetic activity on three different microbial tester systems. These systems are: a) *Escherichia coli*, Strain B (Hill) isogenic series of repair deficient strains, lethality and induction of mutation to streptomycin or D-cycloserine resistance. b) *Salmonella typhimurium*, Strains TA 1535, TA 1537, TA 1538, TA 100 and TA 98, reversion from histidine requirement to histidine prototrophy. c) *Saccharomyces cerevisiae*, Strain D-7, induction of gene conversion at the thr locus.

The fungicides Polyram (ammonia complex of zinc ethylenebis-dithiocarbamate and polyethylenebis (thiocarbamoyl) disulfides) and Dithane M-45 (manganese-zinc ethylenebisdithiocarbamate) were found to cause more gene conversion than Zineb (zinc ethylenebisdithiocarbamate) or Maneb (manganese ethylenebisdithiocarbamate). The genetic activity of Maneb appeared to be markedly different in the two prokaryotic tester systems. In contrast to the weakly mutagenic activity in the five Salmonella tester strains, Maneb induced a five-fold increase of streptomycin resistant mutants in the uvrB− strain of *E. coli* B. Thiram (bis(dimethylthiocarbamoyl)- disulfide) although the most fungitoxic, is only lethal to exr− mutants of *E. coli*.
(Cb-1)
"In Vitro Culture of Mouse Embryos After TEM-treatment of the Fathers." Kurt Burki* and William Sheridan, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.

DBA/2J males were injected i.p. with 0.2 or 0.4 mg/kg TEM in Hanks BSS. Corresponding control animals received simultaneous injections with Hanks BSS only. The males were mated to CD-1 females and these checked for plugs daily. Approximately 32 hours past mating 2-cell embryos were retrieved from the oviducts of the females and established in culture. 72 hours later the embryos were classified and the ones with a blastocoele were transferred into plastic dishes containing a more complex culture medium. Four days later the blastocysts were checked for hatching and outgrowth of the trophoblast.

Treatment of the males did not reduce the rate of fertilized and cleaving eggs but affected further development of the embryos at all stages. The percentages of cultured embryos showing normal development decreased with increasing dose of TEM injected and varied with the time of conception postinjection for treated postmeiotic stages of spermatogenesis. These results are compared with results from corresponding dominant lethal tests. Cytological evidence for disturbances in early embryonic development will be presented.

(Cb-2)
"Predictability of Dominant Lethal Effects from Study of Fertility and General Reproductive Performance (Segment I)." Madhu S. P. Manandhar, and Benjamin A. Jackson, Reproductive Safety, Teratology and Experimental Mutagenicity Group, Lederle Laboratories, Pearl River, New York 10965.

Mutagenicity tests and reproductive studies are routinely conducted to evaluate the reproductive safety of chemicals to which humans are likely to be exposed. Similarity of end points between test systems, could permit utilization of a single test for screening chemicals. This communication focuses on overlapping end points between two currently accepted test systems: the dominant lethal (DL) test and the fertility and general reproductive performance (Segment I) study, (FDA guidelines 1966).

In the DL test, male animals are dosed acutely or subacutely with the test agent and then mated for 8 consecutive weeks to assess the mutagenic potential at every stage of spermatogenesis. In Segment I studies, males are dosed for 60 days (which covers all stages of spermatogenesis) and then mated for 1 or 2 weeks. Therefore, the mating results from a Segment I study show immediately if drug exposure causes DL effects.
Survey of literature with published results on both test systems shows that compounds that were positive in DL test were also positive in Segment I Study while those that were negative also showed a similar correspondence. This observed perfect agreement between the two test systems was expected and demonstrates that the DL effect can be ascertained directly from Segment I studies.

(Cb-3)  "Induction of heritable translocations with methyl methane-sulfonate in male mice."  Rainer Lang and Ilse-Dore Adler, Schering AG, Research Laboratories, Berlin/Bergkamen, and Gesellschaft für Strahlen- und Umweltforschung, München, Germany.

For the evaluation of induced heritable translocations by methyl methanesulfonate (MMS) late spermatids and early spermatocytes were tested. A comparison of fertility data and cytogenetic analyses of the F₁ males was performed to assess the accuracy of identification of semisterile translocation heterozygotes by the reduction of litter size.

NMRI males of proven fertility were injected intra-peritoneally with 40 mg/kg of MMS. 250 F₁ male progeny sired 4 to 10 days after treatment and 245 F₁ control males were tested for their reproductive performance. F₁ males that in three matings produced at least once 10 live implants with none or only one dead and in another two matings at least once a litter of 10 or more live offspring were considered fully fertile. F₁ males that did not meet these criteria were mated again for further fertility testing and to collect F₂ sons from the semisterile males. The testes of all sterile, semisterile and non-classifiable F₁ males as well as those of the semisterile F₂ sons from semisterile F₁ males were prepared for cytogenetic analysis.

A total of 14 semisterile and 14 sterile males (28; 11.2%) were observed in the MMS group. Of these 20 (8.0%) could be identified cytogenetically as heterozygous translocation carriers, i.e. 6 sterile and 14 semisterile males. Heritability of the reciprocal translocation was confirmed for the semisterile F₁ males by cytogenetic identification of translocation carriers among the F₂ progeny. There were no sterile or semisterile males among the 245 F₁ controls.

In assessing the accuracy of classification the frequency of false positives was computed directly from the observed fertility data. Based on 5 matings and applying the rules outlined above it was 16%. Further matings reduced the frequency of false positives to approximately 1%. Since the animals declared normal were not analysed cytogenetically, strictly speaking the probability of false negatives cannot be calculated, however, making certain assumptions a statistical approach is possible.
Mutagenicity in mammals is often revealed when chromosomal aberrations are shown to occur as a result of treatment with suspect materials. Among testing methods presently being used, the heritable translocation protocol appears promising for the detection of such heritable abnormalities. Using the basic test procedure, an attempt was made to simplify the technique, using mice treated with triethylentlenemelamine (TEM), a known mutagenic agent.

Random-bred albino male mice were used for the studies. Four groups of 40 mice each received TEM in drinking water for 4 weeks at the following dosages: 0 (control), 0.0125, 0.025 and 0.05 mg/kg/day. Each male was then bred to 3 virgin females per week for 3 weeks. One female was used to assess the dominant lethal effect of TEM, and other two were allowed to produce the F1 generation. 100-200 F1 males per dose level were raised to maturity and mated to 3 virgin females each. These females were sacrificed at mid-term of pregnancy, and their uterine contents were analyzed for the number of live implants. This number served as an estimation of the fertility of F1 males.

Evaluation of the breeding data shows that the percentages of F1 males with reduced fertility (presumptive translocation heterozygotes) are 2.4, 7.2, 19.2 and 14.3 respectively for control, low, medium and high dosage TEM animals from week-1 mating, and the values are 6.3 and 8.2 respectively for control and high dosage TEM animals from week-3 mating. Week-2 F1 animals were not bred. Meiotic analyses of the presumptive F1 confirm the absence of translocation abnormalities in the control animals. The work on TEM animals is in progress; preliminary examination indicates the presence of such abnormalities.

We have conducted a preliminary investigation into the fate and distribution in mice of triethylentlenemelamine (TEM). TEM (ethyleneimine-1,2',4') with a specific activity of 8.53 mCi/mmol was administered by gavage or by i.p. injection to strain DBA/2J male mice. At one hour and twenty-four hours after injection males...
were killed and the following tissues and materials were prepared for oxidation and analysis: kidneys, testes, vas deferens, blood, urine, and feces. Analyses of the tissues collected from animals killed one hour after injection showed that i.p. injections of TEM had resulted in rapid dispersal of the compound throughout the body. In contrast, levels of radioactivity in tissues of animals treated by gavage were significantly less than those observed for i.p. injected animals.

Consideration of the data obtained 24 hours after injection indicated a rapid turnover and elimination of the TEM, with only a small portion of the label still remaining in the mouse. Comparison between routes of administration at this time showed a significant difference between groups in the amount of label in the urine and feces. In the i.p. injected mice the major portion of the activity was found in the urine with very little label in the feces, while in the mice treated by gavage, the opposite was true.

(Cb-6)
"Unscheduled DNA Synthesis in the Germ Cells of Male Mice Treated In Vivo with Chemical Mutagens Requiring Metabolic Activation." René E. Sotomayor*, Gary A. Sega*, and Robert B. Cumming* Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

Unscheduled DNA synthesis (UDS), taken to be DNA repair, has been observed in meiotic and early to mid spermatid stages of the mouse after in vivo treatment with mitomen and with cyclophosphamide. The more mature germ cell stages, including late spermatids and spermatozoa, do not exhibit a UDS response with either of these chemicals.

Unlike chemical mutagens such as EMS and other alkyl methane-sulfonates which induce a maximum level of UDS immediately after treatment, mitomen and cyclophosphamide induced a maximum UDS response approximately one-half and one hour, respectively, after treatment of the males. This time delay effect may be related to the time required for the metabolic activation of these compounds to active forms.

This research was sponsored by the U. S. Energy Research and Development Administration under contract with the Union Carbide Corporation.

(Cb-7)
In the developing germ cells of male mice the last scheduled DNA synthesis occurs in the preleptotene-S spermatocyte. We have found, however, that later germ cell stages will undergo an unscheduled incorporation of \([^{3}H]\)thymidine (\([^{3}H]dT\)) when exposed in vivo to certain mutagenic agents such as methyl methanesulfonate (MMS) and X-rays.

Male mice were given testicular injections of \([^{3}H]dT\) and then given either a 100 mg/kg dose of MMS or an acute exposure of 600 R of X-rays. After 90 minutes the animals were killed and autoradiographs were made from slide preparations of the germ cells recovered from the testes.

Pachytene stages from males treated with MMS showed the greatest unscheduled DNA synthesis (UDS), with the number of grains over these stages being about 20 to 30 times the control value and 3 to 4 times greater than other treated germ cell stages. Round and elongating spermatids from MMS-treated animals showed about 10 times more labeling than the controls, while late spermatid stages showed very little or no labeling. The same meiotic and postmeiotic germ cell stages that showed UDS after exposure to MMS also exhibited UDS following X-ray treatment although the level of UDS was considerably lower after the X-ray treatment.

This research was sponsored by the U. S. Energy Research and Development Administration under contract with the Union Carbide Corporation.

(Cb-8)

"Comparisons between the Unscheduled DNA Synthesis Induced by Two Alkyl Methanesulfonates and by X-Rays in the Germ Cells of Male Mice." Gary A. Segar* and René E. Sotomayor*, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

Unscheduled DNA synthesis (UDS) has been observed in mouse germ cells from early meiotic stages through early to mid spermatid stages after in vivo treatment with methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and X-rays. Early spermatid stages have been studied in greatest detail using liquid scintillation counting to measure the UDS. For the early spermatids there is a linear increase in UDS as the administered chemical or X-ray dose is increased. Doses as low as 5 mg/kg of MMS, 10 mg/kg of EMS, and 50 R of acute X-rays induced UDS. On the basis of a unit change in chemical dose (\(\Delta 1\) mg/kg) compared to a unit change in X-ray dose (\(\Delta 1\) R), the MMS and EMS were 130 and 30 times as effective, respectively, as the X-rays in inducing UDS in early spermatids. UDS could be detected in early spermatid stages as long as three days after chemical treatment while with 600 R of X-rays UDS was no longer detected 4 to 6 hours after exposure.
Dominant lethals are not observed in early spermatid stages after treatment with MMS or EMS. The most sensitive germ cell stages are spermatozoa and late spermatids which do not exhibit any UDS. However, X-rays induce about twice the dominant lethal frequency in early to mid spermatid stages which exhibit UDS, as they do in later germ cell stages (late spermatids to spermatozoa) which exhibit no UDS. No good evidence, therefore, exists to indicate that UDS has any effect on dominant lethal mutation frequencies in the germ cells of male mice.

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(Cb-9)
"Combined Effects of Methyl Methanesulfonate and X-Rays on Unscheduled DNA Synthesis in Early Spermatids of the Mouse." Donald A. Carpenter* and Gary A. Segar*, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

The unscheduled incorporation of tritiated thymidine ([3H]dT) into certain germ cell stages of male mice after exposure to methyl methanesulfonate and X-rays has already been demonstrated. We report here on the effect of combined treatment with MMS and X-rays on the induction of unscheduled DNA synthesis (UDS) in early spermatids. (C3Hf x 101)F1 males were given intraperitoneal injections of 75 mg/kg of MMS and testicular injections of 78 μCi of [3H]dT per animal, followed immediately by acute X-ray exposures ranging from 50 R to 1200 R. Additional males were first given the various X-ray doses and then, four hours later, given the MMS and the [3H]dT. UDS in early spermatid stages was determined by measuring the [3H]dT activity in caudal sperm 16 days after treatment, using liquid scintillation counting.

Preliminary experiments, using a combination of MMS and 1200 R of X-rays given simultaneously, have shown that while the UDS induced in early spermatids was still five times higher than with the X-rays alone, there was a three-fold reduction in the level of UDS compared to what was obtained using MMS alone. At present these results suggest that X-ray treatment of early spermatids inhibits their UDS response to MMS.

This research was jointly sponsored by the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, and the U. S. Energy Research and Development Administration under contract with the Union Carbide Corporation.
(Cb-10)  

A method for the detection of somatic mutations in the mouse was developed by us in the past (Russell and Major, 1957, Genetics 42: 161-175). We showed that the rate of radiation induction at four specific loci is of the same order of magnitude as that in spermatogonia; that the modal number of prospective pigment cells in 10-1/4-day embryos is 150-200; that an induced increase in ventral white spots can occur as a result of cell killing rather than mutation; and that other nongenetic coat-color effects and dominants do not unduly interfere with detection of somatic mutations.

We are now exploring the usefulness of this method for chemical mutagenesis studies. The major thrust of this investigation is to determine parallelisms to and differences from genetic effects of the same chemicals in the germline.

Embryos heterozygous for four coat color loci and piebald are exposed to the putative mutagen in utero on day 10 1/4 postconception. The animals are subsequently checked at birth, and their coats are carefully examined for colored and white spots on days 12 and 30. We have obtained positive results for 0.8 mg/kg and 0.5 mg/kg of TEM and 2 mg/kg of mitomycin-C, both of which also induce mutations in mouse spermatogonia. To date, results are negative for 50 mg/kg MMS and (in a small series) for 50 mg/kg EMS. Neither of these chemicals have so far shown a clear induction of mutations in spermatogonia. Work with polycyclic hydrocarbons is in progress.

Research sponsored by the U. S. Energy Research and Development Administration under contract with the Union Carbide Corporation.

(Cb-11)  

Previously unreported data from our laboratory and new results obtained elsewhere confirm the earlier conclusion reached by national and international committees that, at low levels of radiation, the genetic risk to the female is considerably less than that to the male. This is in contrast to recent claims (e.g. Wolff, S., Mutation Research, 33: 95-102), based on a theoretical model, that the mutation rate in females from low-dose-rate irradiation should prove to be higher than that in the male.

Research sponsored by U. S. Energy Research and Development Administration under contract with the Union Carbide Corporation.
"Measurement of Mutation at the HGPRT Locus in Diploid Human Lymphoblasts." William G. Thilly, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The interesting physiological characteristics of the relationship of cellular hypoxanthine-guanine phosphoribosyl transferase activity to resistance to 6-thioguanine, which have prevented accurate dose-mutation response determination in human and other mammalian cells, are discussed. The existence of a phenotypic lag period of fourteen generations appears to reflect a requirement for total loss of pre-existing HGPRT molecules before 6TG-resistance is expressed.

Dose-response studies for MNNG, MNU, BUDR, CldUrd, IUDR, and ICR191 show that induced mutant fraction increases with mutagen concentration and is stable under non-selective growth conditions.

"Dose-Response Relationships of Cytotoxicity and Mutagenicity of Mono-functional Alkylating Agents in Chinese Hamster Ovary Cells." David B. Couch and Abraham W. Hsie, The University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

It has been shown in certain microbes that alkylating agents which react through an unimolecular nucleophilic substitution (SN1) mechanism produce relatively more miscoding alterations in DNA than those which react via a bimolecular (SN2) mechanism, since the former react more extensively at the relatively weaker nucleophilic sites, including the O6-atom of guanine. Since alkylation at this position produces mispairing, the mutagenicity of these compounds would be expected to correlate with tendency to react by the SN1 mechanism.

We have studied the biological effects of the alkylating agents methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), dimethylsulfate (DMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), N-methyl-N-nitrosourea (MNU) and N-ethyl-N-nitrosourea (ENU) in Chinese hamster ovary (CHO) cells. These compounds include typical SN2 alkylating agents (DMS, MMS), those which have a greater SN1 character (MNU, MNNG) and those which show intermediate properties (EMS). For each compound single cell survival and induced mutation frequency at the hypoxanthine-guanine phosphoribosyl transferase locus of CHO cells have been determined as a function of dose of alkylating agent. The following order of increasing mutagenicity (induced mutation frequency per unit concentration of mutagen) was observed:
EMS < ENU < DMS < MMS < MNU < ENNG < MNNG. Cytotoxicity followed approximately the same order: EMS < ENU < MMS < DMS, MNU < ENNG < MNNG.

A positive correlation between mutagenicity and known chemical reactivity was not always found. Through the series of alkylating agents DMS to MNNG and MNU both the tendency to react by the $S_{N1}$ mechanism and the mutagenicity increase. However, the ethyl analog of each class of compound studied is known to have greater $S_{N1}$ character than the corresponding methyl derivative, but was found to be less mutagenic. (Research supported by the U.S. Energy Research and Development Administration under contract with the Union Carbide Corporation.) 1 Postdoctoral Investigator supported by Carcinogenesis Training Grant No. CA05296.

(Ea-3)
"Liver-Homogenate Mediated Mutagenesis in Chinese Hamster V79 Cells by Chemical Carcinogens." David F. Krahn* and Charles Heidelberger, McArdle Lab., Univ. of Wis., Madison, WI., 53706

Many chemical carcinogens are not mutagenic prior to conversion to active metabolites by specific mammalian enzymes. In our studies several diverse carcinogens were found to exhibit dose-dependent cytotoxicity and produce an increase in the frequency of thioquanine-resistant (15µg/ml, dialyzed serum) colonies in Chinese hamster cells when incubated in the presence of the 9,000 x g supernatant fraction of rat liver and the cofactors, NADPH and NADH. Alone or in an incomplete incubation mixture these chemicals were inactive. The approximate concentration required to reduce the control plating efficiency by 50%, the fold induction of thioquanine-resistant variants over control levels, and the source of liver homogenate (from rats that received methylcholanganthe (MC), phenobarbital (PB), or were untreated (U)) for some of the carcinogens are as follows: aflatoxin B$_1$ (0.3 nM/ml, 10, U), aflatoxin B$_2$ (35 nM/ml, 2, U), benzo[a]pyrene (16 nM/ml, 10, MC), benz[a]anthracene (30 nM/ml, 4, MC), dimethylnitrosamine (13 µM/ml, 200, PB). Cytotoxicity and an increased frequency of thioquanine-resistant variants under activating conditions has also been observed with 7,12-dimethylbenz-[a]anthracene, methylcholanganthe, and 2-naphthylamine. A study of 4-aminobiphenyl, N-methyl-4-aminooazobenzene, dibenz[a,c]anthracene, and dibenz[a,h]anthracene is in progress. To date all attempts to activate 2-acetylaminofluorene have been futile. Of the thioquanine-resistant colonies examined, all retain the resistant phenotype following prolonged growth in the absence of drug, and are sensitive to HAT. These studies demonstrate that chemical carcinogens that require metabolic activation can be screened for mutagenic activity in cultured mammalian cells that are deficient in mixed-function oxidase activity.

This research is supported in part by grants CA-07175 and CRTY-5002 from the National Cancer Institute.
Considerable effort has been put into the development of in vitro microsomal enzyme "activation systems" for microbial mutagenesis assays. Promutagens are metabolized to active mutagens when incubated in the presence of the microsomal enzyme preparations and the appropriate buffers and cofactors.

The utility of other in vitro mutagenesis assays, particularly those involving cultured mammalian cells have been limited because suitable "activation systems" have not been available. We have successfully adapted a microbial "activation system" to an in vitro mammalian cell mutagenicity assay using the L5178Y (TK<sup>+/−</sup>) mouse lymphoma line (Clive and Spector, Mutation Research, 31:17-29, 1975).

The in vitro "activation system" consists of a 9,000 x g supernatant from mouse liver and kidney plus TPN, Tris buffer, and MgCl<sub>2</sub>. In activation tests, the L5178Y TK<sup>+/−</sup> cells, the promutagens, and the activation system are added to the cloning medium used for mutagen exposure.

The components are incubated for five hours at 37°C. Following exposure, the TK<sup>+/−</sup> cells are washed, incubated for expression of induced mutations, and then assayed for the frequency of TK<sup>−/−</sup> mutants.

Mutagenicity data have been obtained from activation and non-activation tests for 2-acetylaminofluorene, 2,4-diaminotoluene, dimethylnitrosamine, diethylnitrosamine, benzo(a)pyrene, dimethylbenzantracene, and m-phenylenediamine. A summary of the system and the data will be presented.

Sister chromatid exchanges have proved to be highly sensitive indices of chromosome damage and repair in cultured cells exposed to chemicals such as mitomycin C. Exchanges can be detected by optical techniques if sister chromatids are unequally substituted with
5-bromodeoxyuridine (BrdU). We have now adapted this approach for the analysis of sister chromatid exchanges formed in vivo in mouse spermatogonia. BrdU is administered to mice over the duration of one DNA synthesis period, and, after a second round of replication, spermatogonial metaphases are prepared from isolated seminiferous tubules. Sister chromatid differentiation is effected either by 33258 Hoechst fluorescence or subsequent Giemsa staining. A baseline sister chromatid exchange frequency of somewhat less than two per diploid genome was observed in nearly 100 such cells.

As a prototype of a general in vivo mutagenesis test, we have utilized this system to examine the effect of mitomycin C on spermatogonial chromosomes. A single intraperitoneal injection of 0.3 μg/gm body weight mitomycin C prior to the last round of replication induces a three-fold increase in the sister chromatid exchange frequency in spermatogonia exhibiting fewer than 0.1 morphological aberrations per cell. At 0.5 μg/gm mitomycin C, the number of second division metaphases is sharply reduced, although those cells scored exhibited an even higher frequency of exchanges. The present system for analysis of in vivo sister chromatid exchange formation should permit sensitive detection of the impact on germ tissue of a variety of potential mutagens, including those requiring host-mediated metabolic changes for activation.

(EA-6)
"Detection of Sister Chromatid Exchanges in Chick Embryos Exposed to Bromodeoxyuridine." Stephen E. Bloom and T. C. Hsu, Department of Poultry Science, Cornell University, Ithaca, New York 14853 and Department of Biology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

Sister chromatid exchange (SCE) can be studied in great detail when cells are grown in media containing 5-bromodeoxyuridine (BrdU) and metaphase preparations are stained with certain fluorescent dyes and/or Giemsa techniques. Increased rates of SCE have been reported for cultured mammalian cells exposed to known chemical mutagens at concentrations that do not cause gross chromosome aberrations. Thus, SCE analysis appears to be an effective method for detecting the presence of minute amounts of chemical mutagens in cells and chromosomes.

We investigated the possibility of using in vivo systems for SCE analysis through BrdU incorporation. Differential fluorescence of sister chromatids (SCD) and SCE were visualized in chromosomes obtained directly from growing chicken embryos. SCD was obtained
by exposing 3-day embryos to BrdU (12.5-50 μg) in ovo for 26 hours and staining air dried chromosome preparations with 33258 Hoechst. Bright, stable fluorescence and continued SCD were achieved if slides were mounted in McIlvaine's pH 4.4 buffer. Embryo growth, mitotic activity and gross chromosome morphology were not adversely altered by the BrdU treatments. The SCE rate was estimated to be 0.07 SCEs per macrochromosome and 0.75 SCEs per metaphase for two cell cycles. These rates are substantially lower than those reported for in vitro systems. We are currently investigating SCE rates in chick embryos exposed to selected chemical mutagens.

(Ea-7)
"Detection of Sister Chromatid Exchange Following 5-Bromodeoxyuridine Incorporation in the Central Mudminnow." Andrew D. Kligerman and Stephen E. Bloom, Department of Poultry Science, Cornell University, Ithaca, New York 14853.

Various methods ranging from the study of point mutations in prokaryotic organisms to cytogenetics studies in mammalian species have been proposed for assessing the genetic hazards of environmental pollutants. We have been interested in developing the central mudminnow, Umbra limi, as an in vivo model for the investigation of chromosome mutations caused by water-borne chemical pollutants. Recently techniques have been developed to detect sister chromatid exchange (SCE) in various cell culture systems as well as Vicia faba and embryos of Gallus domesticus after 5-bromodeoxyuridine (BrdU) incorporation.

We have recently found that sister chromatid differentiation (SCD) and SCE can be visualized in metaphases of Umbra limi 5 to 6 days after the intraperitoneal injection of BrdU (500 μg/g). SCD was found in the metaphases of the intestines, kidneys, gills, and scale epithelium in varying rates. The intestines had the highest rate of SCD varying from 0 to 60% with a mean of 12.3%. SCE rates were found to be 2.70 exchanges/metaphase and 2.32 exchanges/metaphase in the intestines and gills, respectively, which is much lower than the rates obtained in most in vitro systems. SCE analysis seems to be a more sensitive indicator of genetic damage than the classical chromosome breakage studies, and we believe that SCE analysis in Umbra limi can be a useful tool for the study of water-borne chemical mutagens. Presently, studies are underway in our laboratory to determine what effect known mutagens will have on the SCE rate in Umbra limi.

(Ea-8)
Utilizing a previously reported technique (Evans and Jenkins, 1975) a higher frequency of sister chromatid exchange was observed in short-term human leucocyte cultures exposed to methylazoxymethanol acetate (MAH acetate) as compared to control cultures. This effect occurred in all of 9 individuals from whom the cultures were derived.

Two concentrations of MAH acetate were tested in the blood cultures from each individual. The concentrations sometimes varied from individual to individual since they were determined by means of dose-effect curves employing H\textsuperscript{3}-thymidine (1.9 Ci/mM) as an indicator of blastogenesis. The two concentrations tested for mutagenicity were those that either had no deleterious effect upon tritium incorporation as compared to controls or allowed only half as much label incorporation as compared to controls.

In eight of nine individuals the lower concentration caused a higher incidence of sister chromatid exchange as compared to control cultures. In only one case did the higher concentration of MAH acetate increase the incidence of sister chromatid exchange when compared to the lower dose.

Our results show for the first time the effect of MAH acetate upon human chromosomes indicated by sister chromatid exchange frequency. It also confirms earlier studies on the mutagenicity of MAH (Lacquer, 1964; Kobayashi and Matsumoto, 1964).

This is part of a larger study that will be submitted by L.A.E. in partial fulfillment for the degree of Doctor of Philosophy at Fordham University. Partial support for this work was derived from a grant from the National Fellowships Fund.

(Ea-9)
"Dominant Genetic Damage Induced in Male B6CF\textsubscript{1} Mice by γ-rays, Neutrons, and Alpha Particles." Douglas Grahn, Barbara Frystak,* John J. Russell,* and Arthur Lindenbaum,* Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439.

First reports on the potential genetic hazards associated with low levels of plutonium, while not entirely consistent, have demonstrated a genetic threat to exist, though one of uncertain magnitude. We are examining the short-term genetic aspects of this problem in the mouse using a broad base of comparative radiation exposures. B6CF\textsubscript{1} (C57/BL6J/Anl x BALB/cJ/Anl) male mice are being irradiated with single doses of 60Co γ-rays or fast fission neutrons (0.6–1 MeV mean energy) from the JANUS reactor, 24 weekly fractions of γ-rays or neutrons, continuous γ-irradiation, or plutonium alpha irradiation from internally deposited plutonium. Dominant lethal induction rate, testis weight, sperm count, sperm abnormalities, and chromosome translocations induced in spermatogonia are being measured to provide a matrix of response data for the analysis of relative risks and for the determination of RBE. Preliminary data reveal a single dose n/γ RBE of 5–6 for dominant lethals, testis
weight changes, and sperm abnormalities. With eight to twelve weeks of fractionation, the RBE rises to about 10 at a time when testicular measures of injury and recovery appear to equilibrate at exposures below 50 rads of γ-rays and 5 rads of neutrons per week.

The gonads retain 0.05% of the initial plutonium burden for several months after intravenous injection of a 10 μCi/kg dose of monomeric $^{239}$Pu citrate solution. Average gonad dose level is estimated to be $\sim 1$ rad/week, though the 'gonia would receive about twice that level (Green et al., Nature 255: 77, 1975). Initial dominant lethal tests at an accumulation of 20-25 rads estimate the mutation rate to be $\sim 0.001$/gamete/ rad which is one-half to one-third that following neutron dose accumulations of 7.5-22.5 rads or gamma ray accumulations of 75-360 rads delivered in nine weekly exposures. Genetic damage among the post-meiotic germ cells should be greater in the externally irradiated mice per rad of absorbed energy in the whole gonad which would account for the higher observed mutation rate.

Work performed under the auspices of the U.S Energy Research and Development Administration.

(Eb-1)

Spontaneously occurring pink mutant cells can be observed in blue or purple petals and stamen hairs of Tradescantia plants heterozygous for flower color. Mutation frequencies per hair and per cell are highly correlated but not completely proportional because of clonal differences in number of cells per hair. The spontaneous frequency per hair varied from a low of 0.48 ± 0.15 x 10^{-3} for the putative hybrid clone 02 to a high of 5.21 ± 0.20 x 10^{-3} for the hybrid clone 4430 (T. hirsutiflora x subcaulis), and from 2.15 ± 0.16 to 16.98 ± 1.25 x 10^{-3} per hair for non-hybrid clones of T. bracteata, T. gigantea, T. ohiensis, T. paludosa and T. subcaulis. The pink mutation frequency per 10^5 cells for three clones of T. subcaulis varied from 11.8 to 58.9. Frequencies per 10^5 stamen hair cells varied from 3.65 for one scoring of the putative hybrid clone 02 to 23.0 for young plants of the hybrid clone 4430. For the mutable clone 0106, spontaneous pink mutation frequencies per 10^3 hairs varied from about 8.8 ± 2.15 to 46.0 ± 9.80 and per 10^5 cells from 35.7 to 227.8, depending upon length of time after cuttings were made. The variation in frequencies among clones is assumed to have a genetic basis but within a clone is thought to be influenced by such factors as age of plant or inflorescence, environmental conditions under which the plants are grown, and the year or time of year scoring was done. The variation observed with date of scoring suggests the possibility that an unidentified environmental mutagen(s) may be the causal agent(s).

Research supported in part by ERDA and in part by NIEHS.
(Eb-2)
"Response of Somatic Mutation Frequency in Tradescantia to Exposure Time and Concentration of Gaseous Mutagens." Arnold H. Sparrow and Lloyd A. Schairer, Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

The interaction between time of exposure to and concentration of chemical mutagens determines the extent of the mutagenic response. Hence, such information is helpful in designing exposure schedules which will give the optimal mutation yield. Previous work with short-term DBE exposures of clone 4430 has been extended in the present study up to 144 hours at appropriate concentrations varying from 1 to 100 ppm. The resulting data on pink-celled stamen hair mutation frequencies are expressed as mean values for the peak response period (6-10 days after exposure). Concentration (C)-response curves for each exposure period used show straight line relationships on log-log plots with slopes approaching 2. Since these data show a consistent increase in sensitivity with increasing time (T) of exposure the log-log plot of mutation response vs. T x C also exhibit a straight-line relationship but with a 1.11 slope (Corr. coeff. = 0.94).

The mutation response frequency following the 144-hour (6-day) exposure at 2 ppm of DBE was 5 events per 100 stamen hairs or equivalent to that produced by about 50 rads of x rays. Since previous experiments with clone 4430 have established statistically significant increases in mutation rates of 0.5 per 100 hairs for DBE and 0.1 for x rays, meaningful data should be obtainable at DBE levels as low as 0.1 ppm based on extrapolations of the 144-hour concentration-response and T x C curves. The sensitivity of the Tradescantia test system in this range should make it adaptable to mutagen monitoring at very low levels of known or suspected mutagenic air pollutants.

Research supported in part by the U.S. Energy Research and Development Administration and in part by the National Institute of Environmental Health Sciences.

(Eb-3)
"Dosimetry of Tritiated 1,2-dibromoethane in Floral Tissues of Tradescantia." Charles H. Nauman, Paul J. Klotz and Arnold H. Sparrow, Biology and Applied Science Departments, Brookhaven National Laboratory, Upton, New York 11973.

Inflorescences of Tradescantia clone 4430, heterozygous for flower color, were exposed to various concentrations of gaseous tritiated 1,2-dibromoethane (3H-DBE). The pink somatic mutation dose-response curve from a series of experiments was parallel to that determined previously for non-labeled DBE, but elevated due to the contribution of the beta radiation.

Floral parts were dissected from buds following exposures of from 15 min to 6 hours and analyzed for tissue concentration of 3H-DBE by liquid scintillation counting. The bud tissue concentration of 3H-DBE increased in proportion to the exposure concentration. Sepal, petal, anther, stamen and ovary tissues contained similar amounts of activity per mg, indicating that penetration of the chemical was uniform. Subsequent experiments, only partially analyzed at present, indicate that penetration of the gaseous DBE to inner tissues is rapid.
Tissues from flowers which opened for up to 10 days after exposure retained only slightly declining amounts of radioactivity, indicating that the $^3$H-DDE or $^3$H is tightly bound in the tissues. Autoradiographs of stamen hair tissue from buds show silver grains that are randomly distributed over the nucleus and cytoplasm immediately after exposure and for up to 12 days later. Thus, $^3$H-DDE is not localized within the cell.

Research supported in part by the U. S. Energy Research and Development Administration and in part by the National Institute of Environmental Health Sciences.

(Eb-4)

"Screening for Induced Chromosome Loss and Non-disjunction in Drosophila melanogaster."

Sidney Mittler, Northern Illinois University, DeKalb, Illinois 60115.

Adult Drosophila males $yB/se^6y^+$ are either injected, fed or exposed to the suspected mutagen and then mated every two days to $yw^f$. The appearance of $yw^f$ male, the XO male was a result of a loss of X or Y chromosomes, and the exceptional wild body Bar eye female, XXY indicated a primary non-disjunction event. The brooding method was used to assess the sensitivity of the various stages of spermatogenesis to the possible mutagen. Digitonin, a microtubule disrupting agent, dispersed as 0.2% in 10% ethyl alcohol and fed for 24 hours, did not increase chromosome loss or non-disjunction. Injection of 0.75% digitonin also produced negative results. Acriflavine, proflavin, hydrazine, and N-aminourethane fed to adults also did not affect the loss and non-disjunction of chromosomes. Ethylmethylsulfonate increased XO gametes in all stages of spermatogenesis. 1000R of X-rays induced loss of the X chromosome in 2-4, 4-6, 6-8, 8-10 day broods. The cells in meiosis I represented by 6-8 day brood were the most susceptible.

(Eb-5)


A series of pesticides has been tested for mutagenicity in Drosophila. Results, which will be presented, have been either negative or borderline for both recessive X-linked lethals and chromosome "mutations" (rearrangement, loss and non-disjunction). The question arises whether the compounds (technical grade) are in fact non-mutagenic in
Drosophila or whether the results reflect the inappropriateness of some of the techniques. Known mutagens are thus being tested with our chemical-handling, exposure techniques and genetic schemes, and the results have been revealing. The dose:effect curve for EMS indicates that even such a potent mutagen is difficult to detect at concentrations below 10 ppm. Thus, even if higher concentrations of compounds to be tested are unrealistic in terms of human exposure levels, it is not possible to base a conclusion of non-mutagenicity on results from low-level exposures. When the test compound is toxic at higher concentrations, the approach might be by chronic or fractionated exposure in some cases. In other cases an alternative system is indicated.

Different genetic end-points have been compared, namely visibles at specific loci, chromosome mutations and X-linked recessive lethals. The latter have proved to be the most diagnostic and most practical.

Experiments are in progress to test different genetic schemes, in the attempt to sensitize the X-linked recessive lethal test. They involve use of a structurally rearranged X-chromosome and the scoring for lethals in males lacking a Y-chromosome. Supported by a contract from EPA.

(Eb-6)

"The Effects of Dietary Deficiencies for Choline and Nicotinic Acid on the Sensitivity of Drosophila melanogaster to Mutagenic Treatment."
B. W. Geer and D. L. Reno, Department of Biology, Knox College, Galesburg, Illinois 61401.

A wild-type strain of Drosophila melanogaster was preadapted to modified Sang's medium C by continuous culture under sterile conditions for 14 months. The growth requirements of the strain for choline and nicotinic acid were then defined. Dietary concentrations of 8 μg choline/ml and 0.4 μg nicotinic acid/ml were selected for testing because these dietary levels inhibited larval growth but allowed the test animals to survive to the adult stage at near normal rates.

Two day adult males raised on an optimal diet or diets deficient for choline or nicotinic acid were fed a 5% aqueous sucrose solution containing 10^{-5} M Trenimon for 17 hours. The treated males were mated to successive groups of virgin 4-day old Basc females (4 males to 12 females) to yield three 4-day broods. Nutritonally deficient and proficient males were fed a 5% sucrose solution for 17 hours and mated to Basc females for control purposes. The frequencies of X-chromosome lethal mutations in the gametes of the test males were determined by the standard mating scheme.

The X-chromosome recessive lethal mutation frequencies for the pooled brood 1-3 data were significantly higher for both the Trenimon-treated choline-deficient and nicotinic acid-deficient test males than

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the frequency observed in the pooled brood 1-3 data for Trenimon-treated males raised on an optimal diet. The brood 1 frequencies, representing mutagen-treated mature sperm and late stage spermatids, did not differ between nutritionally deficient and proficient test groups. The mutation frequencies for the brood 2 data, derived from treated early stage spermatids and spermatocytes, and brood 3 data, representing treated spermatogonia, were significantly higher for both choline-deficient and nicotinic acid-deficient test males than males fed an optimal diet. Thus, choline and nicotinic acid dietary deficiencies rendered the early stage gametes of male Drosophila more sensitive to the mutagenic action of Trenimon. The spontaneous X-chromosome recessive lethal mutation rates were not altered by the dietary deficiencies. (Supported by NIH Contract No. NO1-ES-5-2126)

(Eb-7)
"Thiotepa-Induced Effects on Genetic Fitness in Aedes aegypti", Paul H. Rodriguez, Division of Allied Health and Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78285.

Studies were conducted on the genetic fitness effects of thiotepa (N, N', N'') - Triethylene thiophosphoramide) in various populations of Aedes aegypti (L.) mosquitoes. Productivity or the average number of adult progeny surviving in a population per unit time was employed to estimate genetic fitness.

Female adults of a heterozygous laboratory strain (ROCK) were treated orally for five days with 0.001%, 0.0025%, 0.0050% and 0.025% thiotepa. Subsequently, control and treated females were crossed with non-treated males; the F1 females were then backcrossed with F1 males derived from a second control culture.

Based on total F2 productivity over a 3-week test period, the mean number of progeny was significantly decreased with exposure to 0.001% and 0.025% of the chemomutagen. The 0.025% culture performed slightly lower than the control whereas the 0.005% group showed a slight but non-significant increase in productivity. Analyses of weekly mean productivity indicate that significant mean differences with the control were demonstrated for the 0.025% populations during the second and third weeks. Significant mean differences between the 0.001% experimental and control F2 populations occurred during the third week period. Similar experiments are now being conducted to test the genetic fitness effects on other strains and of other chemomutagens.
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"Deuterium Isotope Effects in Mutagenesis by Nitroso Compounds." Rosalie K. Elecropu*, UT Biomedical Graduate School, Biology Division, ORNL, Oak Ridge, Tennessee 37830.

Nitrosamines which have deuterium instead of hydrogen in the position α to the nitroso group have been reported to have reduced activity in carcinogenicity tests. This result implies that the loss of a hydrogen is a limiting step in the reaction mechanism leading to tumor formation.

Mutagenicity tests were undertaken with nitrosamines which require metabolic activation, and with nitrosamides which are directly acting mutagens, to determine the effect of deuterium substitution on the activity of both types of compound.

Two nitrosamides, MNNG and methyl nitrosourea, and two nitrosamines, nitrosomorpholine and dinitroso-piperazine and their deuterium containing analogs, were tested for reversion of a nonsense mutation in the tyr locus of E. coli K12 WU 3610 (tyr-, leu-) under appropriate conditions. Nitrosamines activated by rat liver microsomes, but not nitrosamides, were less active as mutagens when the deuterium atom was present. The results suggest that the metabolic activation of nitrosamines to a mutagenic species involves the loss of hydrogen, a reaction which the nitrosamides, in the absence of enzyme, do not undergo.

Supported by ERDA and an NIGMS Training Grant to the Biomedical Graduate School.
<table>
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<th>SATURDAY</th>
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<td>Evaluation of Industrial Chemicals</td>
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