11TH ANNUAL MEETING
ENVIRONMENTAL MUTAGEN SOCIETY

Program and Abstracts

MARCH 16-19, 1980
Radisson Plaza Nashville
Nashville, Tennessee
PLEASE DON'T FORGET YOUR PROGRAM
AND ABSTRACT BOOKLET

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

—— NOTES ——

1. Abstracts were not requested for symposia.

2. Smoking is not permitted in Session Rooms.

3. There are no designated times for poster presentations. The posters will be set up for the entire meeting and authors will indicate on a small card on the poster when he/she will be present for discussion and questions. Please check for these cards on the posters in which you are interested. The listing of posters appears at the end of the Program, immediately before the abstracts.

4. There are no scheduled coffee breaks during this meeting. Coffee will be set up outside the meeting rooms from 10:00 to 10:30 a.m. and from 3:30 to 4:00 p.m.
ELEVENTH 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 encourage in and sponsor research, study and dissemination of information related to this problem.

OFFICERS OF THE SOCIETY

President — M. L. MENDELSOHN, Lawrence Livermore Laboratory (1980)

Vice President-President-Elect — S. WOLFF, University of California, San Francisco (1980)

Secretary — S. GREEN, Environmental Protection Agency (1980)

Treasurer — A. D. MITCHELL, SRI International (1980)

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Richard J. Burk, Jr.
4720 Montgomery Lane, Suite 506
Bethesda, Maryland 20014
Telephone: (301)654-3080
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PROGRAM COMMITTEE

S. WOLFF, Chairperson

REGISTRATION FEES AND HOURS

FEES:

<table>
<thead>
<tr>
<th>Category</th>
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HOURS:

- **Sunday, March 16**: 3:00 - 9:00 p.m.
- **Monday, March 17**: 8:30 a.m. - 5:00 p.m.
- **Tuesday, March 18**: 8:30 a.m. - 5:00 p.m.
- **Wednesday, March 19**: 8:30 a.m. - 12:00 Noon

GENERAL INFORMATION

**HOTEL TELEPHONE NUMBER**: (615)747-4810

The 11th Annual Meeting of the Environmental Mutagen Society will be held at the Radisson Plaza Nashville Hotel, Two Commerce Place (located at the corner of 4th and Union), Nashville, Tennessee 37239, from March 16th through 19th, 1980. Diane Taub, Associate Administrator, may be contacted at the Registration Desk for any special assistance needed.

**COFFEE BREAKS** - There are no scheduled coffee breaks during this meeting, however, coffee will be set up outside the session rooms from 10:00 - 10:30 a.m. and from 3:30 - 4:00 p.m. on each day of the meeting.
SUSTAINING MEMBERS & CONTRIBUTORS

The Council of the Society may elect a corporation a Sustaining Member as a result of demonstrated and substantiated acts benefiting the Society and its purposes. Contained below is a listing of corporations making both contributions and joining the Society as Sustaining Members during 1979.

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THE FOLLOWING COMPANIES HAVE MADE CONTRIBUTIONS TO THE SOCIETY DURING 1979

KC BIOLOGICAL, INC.
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THE ARTHUR D. LITTLE FOUNDATION
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West Point, PA 19486

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P. O. Box 7929
Philadelphia, PA 19101

USV PHARMACEUTICAL CORPORATION
1 Scarsdale Road
Tuckahoe, NY 10707
SUNDAY, MARCH 16, 1980

3:00 - 9:00 p.m.  REGISTRATION  Cumberland Foyer

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8:00 p.m.  COUNCIL MEETING  Jackson Room

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- NOTES -
MONDAY, MARCH 17, 1980
Shelby
8:45 a.m.

(Aa) COMPARATIVE MUTAGENESIS: SYSTEMS I

Presiding: J. T. MacGregor
USDA Western Regional Research Center, Berkeley

&

M. L. Meltz
University of Texas Health Science Center at San Antonio

8:45 a.m. (Aa-1) RELATIVE CONTRIBUTIONS OF B AND T LYMPHOCYTES IN THE HUMAN PERIPHERAL BLOOD MUTAGEN TEST SYSTEM AS DETERMINED BY CELL SURVIVAL, MITOGENIC STimulation AND INDUCTION OF CHROMOSOME ABERRATIONS BY RADIATION.
M. E. Gaulden and J. L. Schwartz
University of Texas Health Science Center at Dallas

9:00 a.m. (Aa-2) STATISTICAL ANALYSIS OF MUTAGENESIS TEST DATA: Ames/Salmonella, CHO/HGPRT, MOUSE LYMPHOMA TK SYSTEMS.
J. D. Irr and R. D. Sneec*
E. I. du Pont de Nemours & Company

9:15 a.m. (Aa-3) ACTIVITY OF QUERCETIN IN THE MOUSE LYMPHOMA L5178Y TK +/- MUTATION, DNA SINGLE-STRAND BREAK AND BALB/C 3T3 TRANSFORMATION ASSAYS.
M. L. Meltz and J. T. MacGregor
University of Texas Health Science Center at San Antonio and USDA Western Regional Research Center, Berkeley

9:30 a.m. (Aa-4) GENETIC EFFECTS OF FLAVONOIDS IN CHINESE HAMSTER OVARY (CHO) CELLS IN VITRO, AND IN RABBIT LYMPHOCYTES AND MOUSE ERYTHROBLASTS IN VIVO.
J. T. MacGregor, A. V. Carrano and J. H. Carver
USDA Western Regional Research Center, Berkeley and Lawrence Livermore Laboratory

9:45 a.m. (Aa-5) THE RELATIONSHIP BETWEEN EARLY DEATHS AND IMPLANTS IN CONTROL AND MUTAGEN TREATED CD-1 MICE IN DOMINANT LETHAL ASSAYS.
D. Anderson, D. B. McGregor* and T. M. Weight*
ICI Ltd, Alderley Park and Inveresk Research International, Edinburgh

10:00 a.m. (Aa-6) RELATIVE EFFICIENCY OF TWO METHODS USED IN THE MOUSE HERITABLE TRANSLOCATION TEST FOR CLASSIFYING MALE PROGENY.
M. Krishna, K. T. Cain, C. W. Sheu, and W. M. Generoso
Oak Ridge National Laboratory and Food and Drug Administration, Washington
MONDAY

(Aa) COMPARATIVE MUTAGENESIS: SYSTEMS I
continued

10:15 a.m. (Aa-7) POINT (SINGLE GENE) AND VIABLE CHROMOSOMAL
MUTATIONS IN LS178Y/TK+/− CELLS CAN BE READILY DIS-
TINGUISHED AND QUANTITATED.
D. Clive, A. G. Batson*, K. O. Johnson*, N. Turner* and
M. M. Moore-Brown
Wellcome Research Laboratories and EPA, Research Triangle
Park

10:30 a.m. (Aa-8) MUTATION INDUCTION AT MULTIPLE GENE LOCI IN
CHINESE HAMSTER OVARY CELLS: COMPARING THE
THYMIDINE KINASE LOCUS WITH THE AAF, TGF, AND OUAR
MARKERS.
J. H. Carver and G. M. Adair
Lawrence Livermore Laboratory and University of Texas System
Cancer Center, Smithville

10:45 a.m. (Aa-9) COMPARISON OF MUTAGENIC, CONVERTOGENIC AND
RECOMBINOCENIC EFFECTS OF SOME ADENINE ANALOGUES
IN SACCHAROMYCES CEREVISIAE D7.
W. G. Sorenson*, J. P. Simpson* and T. Ong (Introduced by
J. A. Elliott)
DRDS, NIOSH, Morgantown, WV

11:00 a.m. (Aa-10) SPECIES DIFFERENCES AND EFFECT OF INDUCERS
UPON THE METABOLIC ACTIVATION BY N-HYDROXYLATION
OF THE BACTERIAL MUTAGEN, 4-AMINOBIPHENYL (4-ABP).
J. K. Epp, J. C. Turner and R. E. McMahon
The Lilly Research Laboratories

11:15 a.m. (Aa-11) SIMULTANEOUS DETERMINATION OF HISTIDINE
REVERTANTS AND 8-AZAGUANINE MUTANTS IN A SINGLE
CULTURE OF S. TYPHIMURIUM STRAIN TA-100.
J. L. Seed and E. Bueing
Johns Hopkins School of Hygiene and Public Health

11:30 a.m. (Aa-12) THE APPARENT NON-MICROSOMAL ACTIVATION OF
2-ACETYLAMINOFUORENE TO A MUTAGEN IN VITRO.
D. E. McGregor
Inveresk Research International Limited, Edinburgh

11:45 a.m. (Aa-13) MUTAGENS IN FOODS: TEST STRATEGY AND
RESULTS WITH BEVERAGES.
D. R. Stoltz, B. Stavric*, R. Klassen*, R. Bendall* and B.
Junkins*
Health Protection Branch, Ottawa
MONDAY
Brentwood/Franklin
8:45 a.m.

(Ab) MUTAGENIC EFFECTS

Presiding: A. J. Wyrobek
Lawrence Livermore Laboratory

8:45 a.m. (Ab-1) PURIFICATION AND CHARACTERIZATION OF MUTAGENS FORMED FROM THE REACTION OF SPERMIDINE WITH NITRITE.
H. Kong and M. L. Murray
Louisiana State University Medical Center

9:00 a.m. (Ab-2) INDUCTION OF REVERSE MUTATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS.
J. C. Fuscoe, J. P. O'Neill and A. W. Hsie
The University of Tennessee – Oak Ridge Graduate School of Biomedical Sciences and Oak Ridge National Laboratory

9:15 a.m. (Ab-3) MUTAGEN INDUCED REVERSION OF A CHINESE HAMSTER OVARY TRIPLE AUXOTROPH.
R. T. Taylor and R. Wu*
Lawrence Livermore Laboratory

9:30 a.m. (Ab-4) MITOCHONDRIAL DNA SYNTHESIS IN HUMAN LYMPHOCYTES AFTER CONSUMPTION OF NATURALLY OCCURRING NITRATE, NITRITE AND NITROSAMINES.
C. T. Miller, N. P. Sen* and E. Lok*
Health Protection Branch, Ottawa

9:45 a.m. (Ab-5) DETECTION OF N-METHYL-N'-NITRO-N-NITROSOGUANIDINE (MNNG) INDUCED INTESTINAL METAPLASIA IN THE GLANDULAR STOMACH OF RATS.
R. W. Morgan, J. M. Ward* and P. E. Hartman
The Johns Hopkins University and National Cancer Institute

10:00 a.m. (Ab-6) MITOMYCIN C DECREASES SPERM ACROSIN ACTIVITY AND MOTILITY IN MICE.
S. C. Johnson*, G. Ficsor and L. C. Ginsberg*
Western Michigan University

10:15 a.m. (Ab-7) DOSE DEPENDENCE OF FLUORESCENCE VARIABILITY IN DNA STAINED SPERM FROM MUTAGEN EXPOSED MICE.
D. Pinkel*, B. L. Gledhill, S. Lake*, M. A. Van Dilla* and A. J. Wyrobek
Lawrence Livermore Laboratory

10:30 a.m. (Ab-8) DOES CARBARYL INDUCE SPERM SHAPE ABNORMALITIES IN HUMANS?
A. J. Wyrobek, G. Watchmaker*, L. Gordon*, K. Wong*, D. Moore II*, and D. Whorton*
Lawrence Livermore Laboratory and Environmental Health Associates, Berkeley

8
MONDAY
Woodland
8:45 a.m.

(Ac) MUTAGEN TESTING I

Presiding: H. E. Brockman
Illinois State University

&

E. R. Nestmann
Health & Welfare Canada, Ottawa

8:45 a.m.  (Ac-1) ISOLATION AND IDENTIFICATION OF MUTAGENIC POLYCYCLIC AROMATIC AMINES IN SYNTHETIC CRUDE OILS.
C. -h. Ho* and B. R. Clark*
Oak Ridge National Laboratory

9:00 a.m.  (Ac-2) CHARACTERIZATION OF THE MUTAGENS ASSOCIATED WITH DIESEL PARTICLE EMISSIONS.
L. Claxton and J. Huisingh
Environmental Protection Agency, Research Triangle Park

9:15 a.m.  (Ac-3) POTENTIAL MUTAGENIC/CARCINOGENIC ACTIVITY OF DIESEL PARTICULATES.
M. Dukovich*, R. E. Yasbin, S. S. Lestz*, T. H. Risby* and
R. B. Zweidinger*
The Pennsylvania State University

9:30 a.m.  (Ac-4) COMUTAGENICITY OF BISULFITE.
T. G. Rosman and R. G. Mallon*
New York University Institute of Environmental Medicine

9:45 a.m.  (Ac-5) MUTAGENICITY AND CYTOTOXICITY OF HALOETHANES IN THE CHO/HGPT SYSTEM.
E. L. Tan* and A. W. Hsie
The University of Tennessee – Oak Ridge Graduate School of Biomedical Sciences and Oak Ridge National Laboratory

10:00 a.m.  (Ac-6) MUTAGENIC MYCOTOXINS FROM FUSARIUM MONILI-
FORME.
L. F. Bjeldanes and L. A. Weib*
University of California, Berkeley

10:15 a.m.  (Ac-7) MUTAGENICITY IN SALMONELLA OF DYES USED BY DEFENCE PERSONNEL FOR THE DETECTION OF LIQUID CHEMICAL WARFARE AGENTS.
Wheat*
Health Protection Branch and Defence Research Establishment, Ottawa
MONDAY

(Ac) MUTAGEN TESTING I
continued

10:30 a.m. (Ac-8) MUTAGENICITY TESTING WITH L5178Y MOUSE LYMPHOMA CELLS.
A. M. Rogers
Air Force Aerospace Medical Research Laboratory, WPAFB

10:45 a.m. (Ac-9) MUTAGENICITY OF TRENIMON AND 2,3,5,6-TETRAE-
THYLENEIMINO-1,4-BENZOQUINONE (TEB) AT THE ad-3 REGION OF NEUROSPORA CRASSA.
H. E. Brockman, C. Y. Hung, T. M. Ong and F. J. de Serres
Illinois State University and National Institute of Environmental Health Sciences

11:00 a.m. (Ac-10) "LATENT" MUTAGENICITY AND CYTOTOXICITY IN A COMPLEX MIXTURE: BIOASSAY OF COAL GASIFIER CRUDE TARS.
T. Wolff*, T. Hughes, D. Nichols* and A. Kolber
Research Triangle Institute

11:15 a.m. (Ac-11) MUTAGENICITY OF FLY ASH FROM A FLUIDIZED-BED COMBUSTOR DURING START-UP AND STEADY OPERATING CONDITIONS.
H. E. Kubitschek and D. M. Williams*
Argonne National Laboratory

11:30 a.m. (Ac-12) BIOLOGICAL AND CHEMICAL CHARACTERIZATION OF BAGHOUSE FLY ASH FROM A FLUIDIZED BED COMBUSTOR BURNING OIL SHALE.
A. L. Brooks, R. Hanson* and A. Sanchez
Lovelace Inhalation Toxicology Research Institute

11:45 a.m. (Ac-13) MUTAGENICITY STUDIES ON MICE CHRONICALLY EXPOSED TO HALOTHANE.
E. Zeiger, D. Fresza*, J. Guthrie*, J. Allen and H. Mukhtar*
National Institute of Environmental Health Sciences

- NOTES -
MONDAY
Jefferson/Victory
8:45 a.m.

(Ad) SISTER CHROMATID EXCHANGE I

Presiding: S. M. Galloway
Litton Bionetics, Inc.

&

R. R. Schreck
Children's Hospital Medical
Center, Boston

8:45 a.m. (Ad-1) SISTER CHROMATID EXCHANGE INDUCTION BY CARCINOGENS DIRECTLY ACTIVATED IN HTC CELLS.
R. Dean, G. D. Bynum, D. Kram and E. L. Schneider*
Gerontology Research Center, NIA, NIH, Baltimore

9:00 a.m. (Ad-2) SISTER CHROMATID EXCHANGE RESPONSE OF MURINE ALVEOLAR MACROPHAGES, BONE MARROW, AND REGENERATING LIVER CELLS FOLLOWING INHALATION OF URETHANE.
M. Cheng*, M. K. Conner and Y. Alarie*
University of Pittsburgh Graduate School of Public Health

9:15 a.m. (Ad-3) INFLUENCE OF METABOLIC POTENTIAL OVER BENZO (a)PYRENE INDUCED SCE.
R. R. Schreck and S. A. Latt
Children's Hospital Medical Center, Boston

9:30 a.m. (Ad-4) COMPARISON OF BCNU INDUCED SCE IN BONE MARROW CELLS OF AKR/J AND BDF, MICE.
J. A. Biegel, S. S. Boggs* and M. K. Conner
University of Pittsburgh

9:45 a.m. (Ad-5) DEMONSTRATION BY SISTER CHROMATID EXCHANGE OF GENETIC DIFFERENCES IN BENZO(a)PYRENE METABOLISM IN CULTURED MOUSE EMBRYOS AT EARLY GESTATIONAL STAGES.
S. M. Galloway, P. E. Perry*, J. Meneses*, D. W. Nebert* and R. A. Pedersen*
Laboratory of Radiobiology, San Francisco, MRC Cytogenetics Unit, Edinburgh, NIH, and Litton Bionetics, Inc.

10:00 a.m. (Ad-6) DIFFERENTIAL INDUCTION OF SISTER CHROMATID EXCHANGES BY INDIRECT-ACTING MUTAGEN-CARCINOGENS AT EARLY AND LATE STAGES OF EMBRYONIC DEVELOPMENT.
L. A. Todd and S. E. Bloom
Cornell University
MONDAY

(Ad) SISTER CHROMATID EXCHANGE I
continued

10:15 a.m. (Ad-7) IN UTERO ANALYSIS OF SISTER CHROMATID EXCHANGE: DIFFERENTIAL SENSITIVITY OF FETAL TISSUES TO MUTAGENIC DAMAGE.
D. Kram, G. Bynum, G. Senula*, C. Bickings* and E. Schneider*
Gerontology Research Center, NIA, NIH, Baltimore

10:30 a.m. (Ad-8) INDUCTION OF MUTATIONS AND SCEs FOLLOWING S9 ACTIVATION AND DEACTIVATION OF PROMUTAGENS IN CULTURED MAMMALIAN CELLS.
R. T. Okinaka, G. F. Strniste, D. J. Chen and B. J. Barnhart
University of California, Los Alamos Scientific Laboratory

10:45 a.m. (Ad-9) STEROID MODULATION OF SISTER CHROMATID EXCHANGE INDUCTION BY MITOMYCIN C AND UV LIGHT.
C. Bickings* and E. Schneider*
Gerontology Research Center, NIA, NIH, Baltimore

11:00 a.m. (Ad-10) A COMPARISON OF METABOLIC SYSTEMS TO ACTIVATE CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN VITRO FOR SISTER CHROMATID EXCHANGE.
P. S. Sabharwal*, J. P. Wojciechowski and P. Kaur*
Thomas Hunt Morgan School of Biological Sciences, University of Kentucky

11:15 a.m. (Ad-11) COMPARISON OF THE SISTER CHROMATID EXCHANGE RESPONSE OF HUMAN DIPLOID FIBROBLASTS AND CHINESE HAMSTER OVARY CELLS TO DIMETHYLNITROSAMINE, BENZO(A)PYRENE AND MITOMYCIN C.
Children's Hospital of E. Ontario and Department of National Health and Welfare, Ottawa

11:30 a.m. (Ad-12) FRANK CHROMOSOMAL BREAKAGE AND METHYLAZOXYMETHANOL ACETATE.
L. A. Evans*, C. J. Duncan* and E. C. Jenkins
Medgar Evers College of CUNY, Brooklyn, and New York State Institute for Basic Research in Mental Retardation, Staten Island

11:45 a.m. (Ad-13) STANDARDIZATION OF THE SISTER CHROMATID EXCHANGE (SCE) ASSAY: RESULTS WITH THE ANTITUMOR AGENTS MITOMYCIN C (MMC), ADRIAMYCIN AND 7-CON-O-METHYLNAGAROL (7-OMEN).
R. J. Trzoz, V. S. Shu* and B. K. Bhuyan*
The Upjohn Company
MONDAY
Brentwood/Franklin
11:00 a.m.

(Ae) ACTIVATION I

Presiding:  D. M. Zimmer
The Upjohn Company

11:00 a.m.  (Ae-1) ACTIVATION OF PROMUTAGENS TO BACTERIAL MUTAGENS BY LIVER POST-MITOCHONDRIAL (S-9) FRACTIONS AND TWO HEPATOCELLE PREPARATIONS.
The Upjohn Company

11:15 a.m.  (Ae-2) FACTORS INFLUENCING THE MUTAGENICITY OF DIMETHYLNITROSAMINE IN THE SALMONELLA PLATE ASSAY.
M. J. Prival and V. D. Mitchell
Food and Drug Administration

11:30 a.m.  (Ae-3) EFFECT OF S-9 CONCENTRATION ON THE FORMATION OF BENZO(a)PYRENE-DNA ADDUCTS IN THE L5178Y MOUSE LYMPHOMA MUTATION ASSAY.
S. C. Thornton, L. Diamond and W. M. Baird*
The Wistar Institute

11:45 a.m.  (Ae-4) DURATION OF S-9 ACTIVITY.
M. S. Kelley* and J. M. Baden
VA Hospital, Palo Alto

12:00 Noon  (Ae-5) THE EFFECT OF CIRCADIAN RHYTHMS AND OTHER FACTORS AS THEY INFLUENCE THE MICROLESION ASSAY.
National Center for Toxicological Research and University of Arkansas for Medical Sciences

- NOTES -
MONDAY
Shelby
2:00 p.m.

(Ba) SYMPOSIUM I

BASIC CONCEPTS IN MUTATION RESEARCH

Presiding: L. Thompson
Lawrence Livermore Laboratory

2:00 p.m. (Ba-1) QUANTITATIVE MEASURES OF INDUCED
MUTAGENESIS.
R. H. Haynes
York University

2:45 p.m. (Ba-2) DNA EXCISION REPAIR IN MAMMALIAN CELLS.
R. B. Setlow
Brookhaven National Laboratory

3:30 p.m. (Ba-3) MUTAGENESIS IN MAMMALIAN SYSTEMS: PROGRES
& PROSPECTS.
R. S. Gupta
McMaster University, Ontario

4:15 p.m. (Ba-4) ENVIRONMENTAL PROMOTORS: THE USE OF AN
IN VITRO ASSAY FOR THEIR DETECTION AND MECHANISM
OF ACTION.
J. E. Trosko
Michigan State University

SYMPOSIA ARE DESIGNED TO ALLOW FOR APPROXIMATELY 10 MINUTES OF
DISCUSSION BETWEEN SPEAKERS.

- NOTES -
MONDAY

6:00 p.m. WELCOME Woodland

M. M. Mendelsohn
President

** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** **

6:00 p.m. RECEPTION Woodland

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15
(Ca) COMPARATIVE MUTAGENESIS: SYSTEMS II

Presiding: D. E. Amacher
Pfizer Central Research

&

W. M. Generoso
Oak Ridge National Laboratory

8:45 a.m.  (Ca-1) THE GENOTOXIC EFFECT OF ADRIAMYCIN IN SOMATIC AND GERMINAL CELLS OF MICE.
W. Au and T. C. Hsu (Introduced by F. E. Arrighi)
University of Texas System Cancer Center, MD Anderson Hospital and Tumor Institute

9:00 a.m.  (Ca-2) DOMINANT LETHALITY IN XENOPUS LAEVIS: INDUCTION OF DOSE-RELATED CYTOGENETIC, MORPHOLOGIC, AND FUNCTIONAL ABNORMALITIES AMONG THE PROGENY OF TRIETHYLENEMELAMINE (TEM)-TREATED MALES.
R. G. McKinnell, B. T. Kren*, T. D. Byrne*, M. A. Schultheis* and J. W. Schaad, IV*
University of Minnesota

9:15 a.m.  (Ca-3) STUDIES ON INDUCTION OF DOMINANT-LETHAL MUTATION AND HERITABLE TRANSLOCATION WITH ETHYLENE OXIDE IN MALE MICE.
W. M. Generoso, C. W. Sheu and R. M. Gryder
Oak Ridge National Laboratory and Bureau of Foods and National Center for Toxicological Research, FDA, Washington

9:30 a.m.  (Ca-4) SELECTING STOCKS OF MICE FOR THE HERITABLE TRANSLOCATION ASSAY (HTA).
J. B. Bishop, R. L. Kodell* and O. E. Domon*
National Center for Toxicological Research, Jefferson, AR

9:45 a.m.  (Ca-5) MUTAGENICITY AND CYTOTOXICITY OF N-chloropiperidine.
M. A. Bempong and F. Scully*
Norfolk State University

10:00 a.m.  (Ca-6) THE GENETIC ACTIVITY OF PARAFORMALDEHYDE IN THE AMES ASSAY, THE L5178Y MOUSE LYMPHOMA ASSAY, THE CHO SCE ASSAY AND IN AN IN VITRO CHO CHROMOSOME ANALYSIS.
D. Brusick, D. Jagannath, B. Myhr and D. Stetka
Litton Bionetics, Inc.
TUESDAY

(Ca) COMPARATIVE MUTAGENESIS: SYSTEMS II
continued

10:15 a.m.  (Ca-7) STREPTONIGRIN AND CYCLOPHOSPHAMIDE INDUCED CHROMOSOME LESIONS IN SOMATIC CELLS VS OOCYTES OF THE RABBIT.
V. Huff, D. Hutton*, R. DuFrain, L. G. Littlefield and J. Wilmer
Oak Ridge Associated Universities

10:30 a.m.  (Ca-8) CLASTOGENESIS AND SEGREGATIONAL ERRORS VS. SPECIFIC LOCUS MUTATION AND SCE FORMATION: FURTHER EVIDENCE FOR DIVERGENT EFFECTS.
A. Bloom, A. Morishima, S. Bennett, F. Nakamura* and R. Henrich
College of Physicians and Surgeons of Columbia University

10:45 a.m.  (Ca-9) THE DETECTION OF CARCINOGENS FROM SEVERAL CHEMICAL CLASSES AS MUTAGENS IN A MODIFIED L5178Y TK MUTATION ASSAY.
D. E. Amacher and G. N. Turner*
Pfizer Central Research

11:00 a.m.  (Ca-10) DETECTION OF DNA DAMAGING ACTIVITY OF ANTI-TUMOR DRUGS NOT MUTAGENIC IN AMES TEST.
K. Bakshi, M. Neita* and S. K. Dutta
Howard University

11:15 a.m.  (Ca-11) MUTAGENESIS OF COAL FLY ASH LINKED TO A TRACHEAL GRAFT ASSAY FOR CARCINOGENESIS.
C. E. Chrisp and G. L. Fisher
University of California, Davis

11:30 a.m.  (Ca-12) MUTAGENICITY AND CARCINOGENICITY OF NITROSAMIDES.
W. Lijinsky and A. W. Andrews
Frederick Cancer Research Center

11:45 a.m.  (Ca-13) MUTAGENICITY AND DNA DAMAGING EFFECTS OF STRONG METHYLATING AGENTS IN SALMONELLA TYPHIMURIUM.
K. A. Rochefort and J. E. Cummins
University of Western Ontario, London
TUESDAY
Woodland
8:45 a.m.

(Cb) MUTAGENESIS: ANALYSIS

Presiding: R. J. DuFrain
Oak Ridge Associated Universities

&
C. Tong
American Health Foundation

8:45 a.m. (Cb-1) DOSE RESPONSE, DISTRIBUTION, AND DISPERSION ANALYSES OF STREPTONIGRIN INDUCED CYTOGENETIC ABNORMALITIES IN SOMATIC CELLS OF THE RABBIT. R. J. DuFrain, E. L. Frome* and L. G. Littlefield Oak Ridge Associated Universities

9:00 a.m. (Cb-2) PARTITIONING OF EXPOSURE-RESPONSE CURVE INTO EXPOSURE-DOSE CURVE AND DOSE-RESPONSE CURVE. W. R. Lee Louisiana State University

9:15 a.m. (Cb-3) EVALUATING STATISTICAL ANALYSES OF MICROBIAL MUTAGENICITY ASSAYS. K. C. Chu*, K. M. Patel*, R. E. Tarone*, A. H. Lin* and V. C. Dinkel National Cancer Institute, NIH, EG&G/Mason Research Institute and Bureau of Foods, FDA, Washington

9:30 a.m. (Cb-4) A TWO-LESION HYPOTHESIS FOR MUTATION INDUCTION IN HAEMOPHILUS INFLUENZAE BY N-METHYL-N'-NITRO-N'-NITROSOGUANIDINE. R. F. Kimball Oak Ridge National Laboratory

9:45 a.m. (Cb-5) GENETIC FACTORS THAT AFFECT THE SPONTANEOUS RATE OF MUTATION. R. C. Woodruff and J. N. Thompson, Jr.* Bowling Green State University and University of Oklahoma

10:00 a.m. (Cb-6) PRELIMINARY CHARACTERIZATIONS OF THYMIDINE KINASE ISOZYMES IN L5178Y MOUSE LYMPHOMA CELL LINES. K. A. Palmer and P. Voytek Food and Drug Administration and Environmental Protection Agency, Washington

10:15 a.m. (Cb-7) DOSE-RELATED CYTOTOXICITY, TRIFLUOROTHYMIDINE-RESISTANCE, AND COLONY SIZE OVER TIME IN METHYL METHANE SULFONATE-TREATED L5178Y TK+/− CELLS. D. E. Amacher, S. C. Paillet* and V. A. Ray Pfizer Central Research
TUESDAY

(Cb) MUTAGENESIS: ANALYSIS

continued

10:30 a.m. (Cb-8) CELL CYCLE SPECIFIC MUTAGENESIS AT THE HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE LOCUS IN THE ADULT RAT LIVER EPITHELIAL CELL.
C. Tong, M. Fazio* and G. M. Williams
Naylor Dana Institute for Disease Prevention, American Health Foundation

10:45 a.m. (Cb-9) THE INFLUENCE OF AGE ON THE ACTIVATION AND METABOLISM OF AFLATOXIN B1 BY RAT LIVER.
A. Jayaraj*, T. Diller and A. Richardson
Illinois State University

11:00 a.m. (Cb-10) STUDIES OF SPECIFIC LOCUS MUTATION INDUCTION AND EXPRESSION IN DIVISION ARRESTED MAMMALIAN CELL CULTURES (CHO/HGPRT SYSTEM).
J. P. O'Neill
Oak Ridge National Laboratory

11:15 a.m. (Cb-11) MUTATION INDUCTION IN THE CHO/HGPRT SYSTEM BY 8-METHOXYPSORALEN (8-MOP) AND NEAR UV LIGHT.
R. L. Schenley*, J. P. O'Neill and A. W. Hsie (Introduced by P. A. Brimer)
Oak Ridge National Laboratory

11:30 a.m. (Cb-12) ISOLATION AND PARTIAL CHARACTERIZATION OF APHIDICOLIN-RESISTANT CHINESE HAMSTER CELLS.
Michigan State University

11:45 a.m. (Cb-13) CAUSES OF STERILITY IN MALE MICE DERIVED FROM GERM CELLS TREATED IN SPERMATOGENIAL OR POSTSPERMATOGENIAL STAGES WITH CYCLOPHOSPHAMIDE.
N. L. A. Cacheco and E. L. Russell
Oak Ridge National Laboratory

- NOTES -
TUESDAY
Jefferson/Victory
8:45 a.m.

(Cc) SISTER CHROMATID EXCHANGE II

Presiding: A. V. Carrano
Lawrence Livermore
Laboratory

8:45 a.m. (Cc-1) SISTER CHROMATID EXCHANGES IN HUMAN LYMPHOCYTES AFTER G, EXPOSURE TO MITOMYCIN C (MMC): EFFECTS OF CONCENTRATION AND EXPOSURE TIME. L. G. Littlefield, S. P. Colyer* and R. J. DuFrain
Oak Ridge Associated Universities

9:00 a.m. (Cc-2) EFFECT OF SODIUM AZIDE ON SCE'S IN HUMAN LYMPHOCYTES AND CHINESE HAMSTER CELLS. P. Arenaz and R. A. Nilan
Washington State University

9:15 a.m. (Cc-3) SISTER CHROMATID EXCHANGE STUDIES IN PETROLEUM REFINERY WORKERS. A. V. Carrano, L. B. Harrison*, B. H. Mayall*, J. L. Minkler* and F. Cohen*
Lawrence Livermore Laboratory, University of Pennsylvania, and Newark Beth Israel Hospital, New Jersey

9:30 a.m. (Cc-4) CYTOGENETIC ANALYSIS OF INDIVIDUALS WITH AN INHERITED PREDISPOSITION FOR CANCER. R. G. Moon, E. J. Gardner* and J. P. Hughes*
University of Utah Research Institute

- NOTES -
TUESDAY
Brentwood/Franklin
8:45 a.m.

(Cd) MUTAGENIC SYSTEMS: ANALYSIS I

Presiding: P. B. Selby
Oak Ridge National Laboratory

8:45 a.m. (Cd-1) TEST OF THE SENSITIVE-INDICATOR METHOD FOR SCREENING FOR DOMINANT SKELETAL MUTATIONS.
P. B. Selby and S. S. Lee*
Oak Ridge National Laboratory

9:00 a.m. (Cd-2) DETECTION OF SOMATIC MUTATIONS IN HUMAN ERYTHROCYTES -- INITIAL HEMOGLOBIN RESULTS AND DEVELOPMENT OF GLYCOPHORIN A AS A NEW MARKER.
W. L. Bigbee
Lawrence Livermore Laboratory

9:15 a.m. (Cd-3) VARIATION IN THE LEVEL OF α-GLYCEROLPHOSPHATE DEHYDROGENASE IN SINGLE SPERM AFTER TREATMENT WITH PROCARBAZINE.
H. V. Malling, C. P. Ray and J. G. Burkhart
National Institute of Environmental Health Sciences

9:30 a.m. (Cd-4) SURVEY OF ESTUARINE SPECIES AS BIOACCUMULATORS OF MUTAGENS.
J. R. Bayliss, T. H. Sparks* and C. W. Chang*
University of West Florida

9:45 a.m. (Cd-5) CHEMICAL MUTAGENESIS IN THE NEMATODE, CAENORHABDITIS ELEGANS.
K. K. Lew, D. Bilodeau*, C. Harding* and E. Zentz*
Children's Hospital Medical Center, Boston, and Harvard Medical School

10:00 a.m. (Cd-6) MUTAGENICITY DETERMINATIONS OF VOLATILE SUBSTANCES USING A MODIFICATION OF THE Ames/SALMONELLA REVERSE MUTATION BIOASSAY.
T. Wolff*, M. Hall*, L. Hildreth* and A. Kolber (Introduced by L. W. Little)
Research Triangle Institute

10:15 a.m. (Cd-7) QUANTITATION OF MAMMALIAN CELL RECOVERIES IN THE PERITONEAL HOST-MEDIATED ASSAY.
University of Texas Medical Branch, Galveston
TUESDAY
Jefferson/Victory
10:00 a.m.

(Ce) REPAIR

Presiding: G. M. Adair
The University of Texas
System Cancer Center,
Smithville

10:00 a.m. (Ce-1) MEASURING SUBTLE DIFFERENCES IN HUMAN DNA REPAIR SYSTEM AND POSSIBLE CORRELATION WITH COLONY FORMING ABILITY.
D. F. Minka, P. Yu* and R. M. Antley
Indiana University

10:15 a.m. (Ce-2) REPAIR OF UV DAMAGE IN HUMAN CELLS ALSO EXPOSED TO AGENTS CAUSING CROSSLINKS, MONO- ADDUCTS, AND ALKYLATEDS.
D. C. Gruenert and J. E. Cleaver*
University of California, San Francisco

10:30 a.m. (Ce-3) DNA REPAIR AND MUTAGENESIS IN UV LIGHT- HYPERSENSITIVE MUTANTS OF CHO-AT3-2 CELLS.
G. M. Adair and J. M. Clarkson*
The University of Texas System Cancer Center, Smithville

10:45 a.m. (Ce-4) TOXICITY AND MUTAGENICITY OF SHALE OIL RETORT PRODUCT WATERS PHOTACTIVATED BY NEAR ULTRAVIOLET LIGHT.
G. F. Stnmiste and R. J. Brake*
University of California, Los Alamos Scientific Laboratory

11:00 a.m. (Ce-5) COMPARISON OF THE EFFECT OF CHEMICAL MUT- AGENTS IN REPAIR-DEFICIENT AND SUFFICIENT AD-3 MUTANTS OF NEUROSPORA CRASSA.
J. O. Converse*, J. D. Stewart* and T. Ong
DRDS, NIOSH, Morgantown, WV

11:15 a.m. (Ce-6) ROLE OF DNA REPAIR IN UV RADIATION AND CHEMICAL MUTAGENESIS IN CHO CELLS STUDIED WITH REPAIR-DEFICIENT MUTANTS.
L. H. Thompson, K. W. Brookman* and C. L. Mooney*
Lawrence Livermore Laboratory

11:30 a.m. (Ce-7) SYNERGISM OF EMS AND MNNG MUTAGENESIS IN EXCISION REPAIR DEFECTIVE BACTERIA AND HUMAN CELLS.
R. Mandel, G. Zuerndorfer* and R. M. Baker
Boston University School of Medicine and Massachusetts Institute of Technology
11:45 a.m.  (Ce-8) ENHANCED SUSCEPTIBILITY OF A XERODERMA PIGMENTOSUM CELL LINE TO MUTAGENESIS BY MNNG AND EMS.  
R. M. Baker, G. Zuerndorfer* and R. Mandel  
Massachusetts Institute of Technology and Boston University School of Medicine

12:00 Noon  (Ce-9) A COMPARATIVE EVALUATION OF MICROSPERMAN SION MICROBIAL DNA REPAIR SYSTEMS.  
N. E. McCarroll, B. H. Keech* and C. E. Piper  
Hazleton Laboratories America, Inc.
TUESDAY
Brentwood/Franklin
10:45 a.m.

(Cf) PHYSICAL MUTAGENS

Presiding: A. L. Carsten
Brookhaven National Laboratory

10:45 a.m. (Cf-1) INDUCTION OF GENERALIZED CHROMOSOME SHATTERING AND MICRONUCLEUS FORMATION BY ULTRAVIOLET LIGHT AND CAFFEINE: ANTAGONISTIC EFFECT OF DEOXYRIBONUCLEOSIDES.
C. Cremer, T. Cremer and M. Simickova
University of Freiburg and University of Heidelberg, Federal Republic of Germany (C. Cremer presently at Lawrence Livermore Laboratory)

11:00 a.m. (Cf-2) UV DOSE DEPENDENT INDUCTION OF ELECTROPHORETICALLY DETECTABLE MUTANTS IN CHO CELLS.
M. J. Siciliano and R. M. Humphrey
The University of Texas System Cancer Center at Houston and Smithville

11:15 a.m. (Cf-3) DNA DOUBLE STRAND BREAKS IN MAMMALIAN CELLS AS MEASURED BY NEUTRAL FILTER ELUTION.
M. O. Bradley
Merck Institute for Therapeutic Research

11:30 a.m. (Cf-4) EFFECT OF X-RAY DOSE FRACTIONATION ON THE FREQUENCY OF RECESSIVE MUTATIONS INDUCED IN OOGONIA OF DROSOPHILA.
H. U. Meyer and S. Abrahamson
University of Wisconsin

11:45 a.m. (Cf-5) POSITION-SPECIFIC EFFECTS OF TRITIUM DECAY IN MATURE SPERM.
W. R. Lee and P. M. S. Skinner
Louisiana State University

12:00 Noon (Cf-6) MUTAGENICITY OF HEAT AT THE AD-3 REGION OF NEUROSPORA CRASSA.
M. D. Anderson
Illinois State University

- NOTES -
TUESDAY

2:00 p.m.  ANNUAL BUSINESS MEETING  Woodland
&
AWARDS

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- NOTES -
TUESDAY
Woodland
3:30 p.m.

(Da) SYMPOSIUM II

THE CHEMISTRY OF ENVIRONMENTAL MUTAGENESIS

Presiding: J. W. Drake
NIHES

--- 3:30 p.m.  (Da-1) FORMATION AND PERSISTENCE OF ALKYL
DERIVATIVES IN MAMMALIAN NUCLEIC ACIDS AS CON-
TRIBUTING FACTORS IN MUTAGENESIS.
B. Singer
University of California, Berkeley

4:15 p.m.  (Da-2) STRUCTURAL AND FUNCTIONAL DAMAGE IN DNA
INDUCED BY ENVIRONMENTAL CARCINOGENS. (With Film)
D. Grunberger
Columbia University College of Physicians and Surgeons

5:00 p.m.  (Da-3) THE ROLE OF DNA-GLYCOSYLASE IN THE INITIAL
STEP OF REPAIR OF PYRIMIDINE DIMERS.
R. H. Grafstrom
The Johns Hopkins University School of Hygiene & Public
Health

SYMPOSIA ARE DESIGNED TO ALLOW FOR APPROXIMATELY 10 MINUTES OF
DISCUSSION BETWEEN SPEAKERS.

NOTE: Dr. Grunberger has consented to show the new version of his
computer programmed film on "The Interaction of Carcinogens
with Nucleic Acids" as a part of his presentation.

- NOTES -

26
WEDNESDAY, MARCH 19, 1980
Jefferson/Victory
8:45 a.m.

(Ea) COMPARATIVE MUTAGENESIS: CHEMICALS

Presiding: G. A. Sega
Oak Ridge National Laboratory

8:45 a.m. (Ea-1) EFFECT OF EXTRACTS OF MUTAGENIC AND NON-
MUTAGENIC FLY ASH ON MAMMALIAN CELL DNA.
T. J. Facklam, J. P. Crowley and M. A. Drum*
Battelle Memorial Institute

9:00 a.m. (Ea-2) CYTOTOXICITY AND MUTAGENICITY OF DIESEL
EXHAUST SOOT EXTRACTS.
A. P. Li, A. L. Brooks and R. E. Royer*
Lovelace Inhalation Toxicology Research Institute

9:15 a.m. (Ea-3) COMPARATIVE MUTAGENICITIES OF METHYLETHYL-
NITROSAMINE, DIMETHYLNITROSAMINE AND DIETHYLNIT-
ROSAMINE.
J. B. Guttenplan
NYU Dental Center

9:30 a.m. (Ea-4) ADDITIONAL RESULTS FROM SPECIFIC-LOCUS
TESTS ON THE SUPERMUTAGENICITY OF ETHYLNITRO-
SUREA IN THE MOUSE.
W. L. Russell, P. R. Hunsicker* and D. A. Carpenter*
Oak Ridge National Laboratory

9:45 a.m. (Ea-5) CHEMICAL DOSIMETRY AND UNSCHEDULED DNA
SYNTHESIS STUDIES OF ETHYLENE DIBROMIDE IN THE
GERM CELLS OF MALE MICE.
G. A. Sega and R. E. Sotomayor
Oak Ridge National Laboratory

10:00 a.m. (Ea-6) BENZENE METABOLITES INCREASE SISTER CHROM-
ATID EXCHANGES AND DISTURB CELL DIVISION KINETICS
IN HUMAN LYMPHOCYTES.
K. Morimoto and S. Wolff
University of California, San Francisco

- NOTES -

27
WEDNESDAY
Shelby
8:45 a.m.

(Eb) MUTAGENIC SYSTEMS: ANALYSIS II

Presiding: J. D. Irr
E. I. du Pont de Nemours
& Company

&
K. L. McCarthy
Rohm & Haas Company

8:45 a.m. (Eb-1) NEUROBLASTS OF THE GRASSHOPPER EMBRYO AS A NEW MUTAGEN TEST SYSTEM.
J. C. Liang and M. E. Gaulden
University of Texas Health Science Center at Dallas

9:00 a.m. (Eb-2) CLUSTERING OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN MULLER-5 TESTS IN DROSOPHILA.
A. J. Katz
Illinois State University

9:15 a.m. (Eb-3) AN ASSAY FOR THE DETECTION OF INDUCED ANEUPLOIDY AND CHROMOSOME BREAKAGE IN DROSOPHILA.
P. A. Foureman
University of Wisconsin

9:30 a.m. (Eb-4) A MUTANT IN DROSOPHILA WHICH INCREASES THE FREQUENCY OF DISOMIC EGGS.
J. M. Mason (Introduced by R. Sharma)
National Institutes of Environmental Health Sciences

9:45 a.m. (Eb-5) FURTHER DEVELOPMENT AND VALIDATION OF A BIOCHEMICAL PROPHAGE INDUCTION ASSAY FOR CARCINOGENS.
R. K. Elespuru and R. W. Pennington*
Frederick Cancer Research Center

10:00 a.m. (Eb-6) USE OF E. COLI K-12 (343/113) FOR THE DETECTION OF MUTAGENIC ACTIVITY INDUCED BY ALIPHATIC NITRO-SAMINES.
T. K. Rao, B. E. Allen, W. Winton* and J. L. Epler
Oak Ridge National Laboratory

10:15 a.m. (Eb-7) A PRIMARY HEPATOCYTE ACTIVATION SYSTEM FOR IN VITRO CYTOGENETIC STUDIES.
H. E. Holden, P. A. Crider* and E. H. O'Brien*
Pfizer Central Research

10:30 a.m. (Eb-8) DETECTION OF UNSCHEDULED DNA SYNTHESIS IN RAT LYMPHOCYTES TREATED IN VIVO WITH CYCLOPHOSPHAMIDE AND TRIETHYLENEMELAMINE.
M. J. Skinner, B. DeCastro* and J. F. Eyre*
McNeil Laboratories
(Eb) MUTAGENIC SYSTEMS: ANALYSIS II
continued

10:45 a.m. (Eb-9) MUTAGENICITY OF N-SUBSTITUTED ARYL COM-
POUNDS AT THE HYPOXANTHINE GUANINE PHOSPHORIBO-
SYL TRANSFERASE LOCUS IN ADULT RAT LIVER
EPITHELIAL CELLS.
G. M. Williams, S. Telang* and C. Tong
Naylor Dana Institute for Disease Prevention, American
Health Foundation

11:00 a.m. (Eb-10) 2-DEOXYGALACTOSE RESISTANCE IN CHINESE
HAMSTER OVARY CELLS.
M. J. Grant and J. D. Irw
E. I. du Pont de Nemours & Company

11:15 a.m. (Eb-11) DEVELOPMENT OF PRACTICAL METHODOLOGY
FOR THE USE OF THE C3H 10T1/2 CELL TRANSFORMATION
ASSAY IN AN INDUSTRIAL LABORATORY.
K. L. McCarthy and H. E. Scribner
Rohm and Haas Company

11:30 a.m. (Eb-12) MORPHOLOGICAL TRANSFORMATION OF C3H/10T1
CL3 CELLS BY ALKYLATING AGENTS.
Y. Oshiro, P. Balwierz and S. V. Molinary
G. D. Searle & Company

11:45 a.m. (Eb-13) CONDITIONS FOR QUANTIFYING MUTAGENICITY
OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE CHO/
HGPRT ASSAY.
R. Machanoff, J. P. O'Neill, J. S. San Sebastian, P. A.
Brimer and A. W. Hsie
Oak Ridge National Laboratory

- NOTES -
(Ec) MUTAGEN TESTING II

Presiding: T. H. Connor
University of Texas
Medical Branch, Galveston

&

G. R. Warren
Montana State University

8:45 a.m.  (Ec-1) MUTAGENICITY OF N-NITROSOPYRROLIDINE
DERIVITIVES IN THE SALMONELLA/MAMMALIAN MICROsome
ASSAY,
Oak Ridge National Laboratory

9:00 a.m.  (Ec-2) MUTAGENICITY OF N-NITROSOPYRROLIDINE
DERIVATIVES IN SACCHAROMYCES CEREVISIAE.
F. W. Larimer, A. A. Hardigree, L. R. Dry* and J. L.
Epler
Oak Ridge National Laboratory

9:15 a.m.  (Ec-3) MUTAGENICITY OF DIETHYLSTILBOESTROL IN
SACCHAROMYCES CEREVISIAE.
R. D. Mehta and R. C. von Borstel
University of Alberta, Edmonton

9:30 a.m.  (Ec-4) MUTAGENICITY OF METAL SALTS IN THE L5178Y
MOUSE LYMPHOMA ASSAY.
T. J. Oberly and C. E. Piper
Hazleton Laboratories America, Inc.

9:45 a.m.  (Ec-5) BACTERIAL MICROsuspension ASSAYS WITH
BENZENE AND OTHER ORGANIC SOLVENTS.
N. E. McCarroll, C. E. Piper and B. H. Keech*
Hazleton Laboratories America, Inc.

10:00 a.m.  (Ec-6) MUTAGEN TESTING OF URINE SAMPLES FROM
SCHOOL CHILDREN IN AN ISOLATED HIGH LUNG CANCER
MODALITY AREA.
G. R. Warren and S. J. Rogers
Montana State University

10:15 a.m.  (Ec-7) REVERSE PHASE HPLC ANALYSIS OF MUTAGENIC
ACTIVITY IN CIGARETTE SMOKERS' URINE.
R. M. Putzrath* and E. Eisenstadt
Harvard School of Public Health

10:30 a.m.  (Ec-8) THE MUTAGENICITY OF FRACTIONATED ORGANICS
EXTRACTED FROM AMBIENT AIR.
A. Kolber, T. Hughes, T. Wolff*, M. Waters, L. Claxton
and J. Huisingh
Research Triangle Institute and US EPA, RTP
(Ec) MUTAGEN TESTING II
continued

10:45 a.m. (Ec-9) THE MUTAGENICITY OF DIALKYLAMINOALKYL CHLORIDES IN A BATTERY OF THREE SHORT-TERM ASSAYS. C. Thompson, S. Rinzel, G. Probst and R. E. McMahon Lilly Research Laboratories

11:00 a.m. (Ec-10) IN VITRO MUTAGENICITY AND TRANSFORMING ACTIVITY OF ELECTROSTATIC COPY IMAGER. T. H. Connor and J. B. Ward, Jr. University of Texas Medical Branch, Galveston

11:15 a.m. (Ec-11) ETHYLNITROSOUREA IS HIGHLY POTENT IN THE MAMMALIAN SPOT TEST. L. B. Russell and C. S. Montgomery* Oak Ridge National Laboratory

11:30 a.m. (Ec-12) EVALUATION OF THE MUTAGENICITY OF n-BGE AND t-BGE IN A BATTERY OF SHORT-TERM ASSAYS. T. H. Connor, T. G. Pullin, J. Meyne*, A. F. Frost* and M. S. Legator University of Texas Medical Branch, Galveston

11:45 a.m. (Ec-13) ACTIVATION OF THE LDH-C GENE IN HEPATOMOCYES OF DBA/2J MICE. J. G. Burkhart*, H. V. Malling and C-Y. Lee National Institute of Environmental Health Sciences
(Ed) PLANT SYSTEMS

Presiding: T-H. Ma
Western Illinois University
&
H. N. B. Gopalan
University of Nairobi,
Kenya

8:45 a.m. (Ed-1) TESTS FOR MUTAGENICITY OF LAKE BLOOMINGTON WATER IN SALMONELLA AND MAIZE.
Illinois State University, Normal, University of Illinois, Urbana, and University of Maryland, Solomons

9:00 a.m. (Ed-2) DIFFERENT MUTATIONAL RESPONSE OF ZEA AND TRADESCANTIA TO ENVIRONMENTAL POLLUTION.
W. R. Lower
University of Missouri Environmental Trace Substances Research Center

9:15 a.m. (Ed-3) FORWARD MUTATION IN INBRED EARLY-EARLY SYNTHETIC, Zea mays BY CHRONIC EXPOSURE TO EMS.
M. J. Plewa, M. L. Ho*, P. A. Dowd* and E. D. Wagner*
University of Illinois

9:30 a.m. (Ed-4) ZEA MAYS AS A MONITOR FOR IN SITU MUTAGENESIS.
V. K. Drobney*
University of Missouri Environmental Trace Substances Research Center

9:45 a.m. (Ed-5) CHROMOSOME BREAKAGE INDUCED BY MALEIC HYDRAZIDE IN CULTURED HUMAN LYMPHOCYTES AND TRADESCANTIA POLLEN MOTHER CELLS.
I. Ahmed and T-H. Ma
Western Illinois University

10:00 a.m. (Ed-6) IN SITU MONITORING OF AIR POLLUTANTS AND SCREENING OF CHEMICAL MUTAGENS USING TRADESCANTIA MICRONUCLEUS BIOASSAY.
T-H. Ma, V. A. Anderson* and I. Ahmed
Western Illinois University

10:15 a.m. (Ed-7) A PROBE OF MUTAGENICITY WITH THE PLANT ARABIDOPSIS.
G. P. Rédei (Introduced by A. Eisenstark)
University of Missouri
WEDNESDAY

(Ed) PLANT SYSTEMS
continued

10:30 a.m. (Ed-8) DETERMINATION OF BIOCHEMICAL AND PHYSIOLOGIC EFFECTS OF ENVIRONMENTAL CONTAMINANTS USING GLYCINE MAX.
D. L. Batema (Introduced by S. Sandhu)
University of Missouri Environmental Trace Substances Research Center

10:45 a.m. (Ed-9) RADIATION-INDUCED MORPHOLOGICAL MUTATIONS IN HAworthia Mirabilis CALLUS TISSUES.
K. N. Pandey*, P. S. Kahlon, P. S. Sabharwal* and J. Calkins*
Tennessee State University and University of Kentucky

11:00 a.m. (Ed-10) CYTOLOGICAL STUDIES ON METHYLENE CHLORIDE.
H. N. B. Gopalan, G. D. E. Njagi* and P. Hongo-Allego*
University of Nairobi, Kenya

11:15 a.m. (Ed-11) QUANTITATION OF MUTAGENESIS IN PLANT CELL CULTURES.
G. Weber and K. G. Lark (Introduced by P. Hynds)
University of Utah

- NOTES -
WEDNESDAY
Jefferson/Victory
10:45 a.m.

(Ee) ACTIVATION II

Presiding: G. R. Douglas
Department of National
Health & Welfare, Ottawa

10:45 a.m. (Ee-1) EFFECT OF IMPURITIES AND METABOLIC
ACTIVATION ON THE MUTAGENIC ACTIVITY OF
RHODAMINE B IN BACTERIAL AND MAMMALIAN CELLS.
G. R. Douglas, C. E. Grant, R. D. L. Bell, J. M. Wytsma,
E. R. Nestmann and D. J. Kowbel
Department of National Health and Welfare, Ottawa

11:00 a.m. (Ee-2) MUTATION FREQUENCIES OF SOME STANDARD
GENETIC TOXICANTS OBTAINED WITH THE CHO/HGPRT
SYSTEM EMPLOYING LOW-SERUM MEDIA AND VARIOUS
CONDITIONS FOR EXOGENOUS (S-9) METABOLISM.
A. R. Malcolm, K. O. Cooper and A. P. Agins*
US EPA, Narragansett, RI

11:15 a.m. (Ee-3) ENHANCEMENT BY GLUTATHIONE OF THE MUTA-
GENICITY OF SELENIUM COMPOUNDS IN MAMMALIAN CELLS.
R. F. Whiting, L. Wei and H. F. Stich
B. C. Cancer Research Centre, Vancouver

11:30 a.m. (Ee-4) MUTAGENICITY AND ENHANCEMENT OF MUTA-
GENESIS BY 2,3,7,8-TETRACHLORODIBENZOFLURAN.
R. Schoeny
University of Cincinnati Medical School

11:45 a.m. (Ee-5) ANDROGENIC REGULATION OF THE ACTIVATION
OF DIMETHYLNITROSAMINE BY KIDNEY MICROSMAL
ENZYMES OF BABL/C MICE.
F. R. Apsy, D. R. Jagannath and S. Mohla*
Howard University

- NOTES -
(Fa) SYMPOSIUM III

INDUSTRIAL SYMPOSIUM
TESTS FOR DETERMINING RISKS FROM ENVIRONMENTAL MUTAGENS

Presiding:  V. Simmon
Genex Corporation

2:00 p.m.  (Fa-1) PUBLIC POLICY PERSPECTIVES OF AN ENVIRONMENTAL DISASTER.
D. Axelrod
Commissioner of Health, State of New York

3:00 p.m.  (Fa-2) RESULTS OF THE INTERNATIONAL PROGRAM FOR THE EVALUATION OF SHORT-TERM TESTS FOR CARCINOGENICITY.
J. Ashby
Imperial Chemical Industries, Ltd.

4:00 p.m.  (Fa-3) STATUS OF THE EPA'S OFFICE OF TESTING AND EVALUATION GENE-TOX PROGRAM.
S. Green
Environmental Protection Agency, Washington

4:45 p.m.  (Fa-4) UTILIZATION OF MUTAGENIC TEST SYSTEMS IN HUMAN MEDICAL MONITORING.
D. J. Kilian
University of Texas at Houston

- NOTES -
WEDNESDAY

8:00 p.m.  COUNCIL MEETING  Jackson Room

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- NOTES -
POSTER PRESENTATIONS

There are no designated times for Poster Presentations. The Posters will be set up in a room separate from the meeting rooms, and will be left up throughout the Meeting. The listing below is numbered and the posters will be set up at the Meeting in this order to make locating a particular poster easier. The authors have been instructed to indicate on a small card on the poster one morning, afternoon, or evening when they will be present for discussion. Please check for these times on the posters that interest you.

P-1 SENSITIVITY OF DROSOPHILA MELANOGASTER TO LOW CONCENTRATIONS OF GASEOUS MUTAGENS: III. DOSE-RATE EFFECTS.
P. G. Kale and J. W. Baum
Brookhaven National Laboratory

P-2 NEUTRON-INDUCED SPECIFIC LOCUS AND X-LINKED LETHAL MUTATIONS IN DROSOPHILA.
S. Abrahamson, C. DeJongh and S. Marino*
University of Wisconsin

P-3 GAMMA-RAY INDUCTION OF HPRT DEFICIENT MUTANTS OF HUMAN LYMPHOBLASTOID CELLS.
H. F. Thomas, R. Rudersdorf* and R. DeMars
The University of Wisconsin

P-4 MUTAGENICITY TESTING USING THE L5178Y/TK+/− MOUSE LYMPHOMA FORWARD MUTATION ASSAY; COMPARISON OF ACTIVITIES OF METABOLIC ACTIVATION SYSTEMS.
D. T. Tajiri and A. D. Mitchell
SRI International

P-5 CYTOGENETIC ANALYSIS OF THE L5178Y MOUSE LYMPHOMA MUTAGENESIS ASSAY SYSTEM.
J. Hozier*, M. Moore-Brown, B. Howard*, T. Danzyl*, and D. Clive
Florida Institute of Technology; EPA, Research Triangle Park; University of Minnesota; and Wellcome Research Laboratories

P-6 MUTAGENIC AND CYTOTOXIC EFFECTS OF HEXAVALENT AND TRIVALENT CHROMIUM IN THE CHO/HGPRT SYSTEM.
K. R. Tindall and A. W. Hsie
The University of Tennessee Oak Ridge Graduate School of Biomedical Sciences and Oak Ridge National Laboratory

P-7 QUANTITATION OF MUTATIONS INDUCED BY METABOLICALLY ACTIVATED MUTAGENS IN NORMAL HUMAN FIBROBLASTS.
D. J. Chen, R. T. Okinaka, and B. J. Barnhart
University of California, Los Alamos Scientific Laboratory
P-8  CONCURRENT ASSESSMENT OF BALB/c 3T3 CELL TOXICITY, MUTAGENICITY AND NEOPlASTIC TRANSFORMATION IN THE PRESENCE OF HEPATIC S-9.
Microbiological Associates

P-9  NEOPlASTIC TRANSFORMATION AND MUTAGENESIS OF BALB/c 3T3 CELLS BY EXTRACTS OF DIESEL AND RELATED ENVIRONMENTAL EMISSIONS.
Microbiological Associates

P-10  IN VITRO MUTAGENESIS AND SISTER CHROMATID EXCHANGE EVALUATIONS OF PARTICLE-BOUND ORGANICS FROM COMBUSTION SOURCES.
E. L. Evans, M. M. Jotz, A. D. Mitchell, J. L. Huisingh and M. M. Moore
SRI International and US EPA, Research Triangle Park

P-11  DEVELOPMENT OF IN VIVO GERMINAL MUTATION SYSTEM USING MONOSPECIFIC ANTIBODY AGAINST SPERM SPECIFIC LACTATE DEHYDROGENASE: SUCCESSFUL DETECTION OF PRESUMPTIVE MUTANT SPERM FROM MICE TREATED WITH PROCARBAZINE.
A. A. Ansari, M. A. Baig* and H. B. Malling
NIH

P-12  DEVELOPMENT OF IN VIVO SOMATIC MUTATION SYSTEM USING HEMOGLOBIN VARIANTS OF MOUSE: PREPARATION AND USE OF ANTI-S HEMOGLOBIN ANTIBODY IN THE DETECTION OF RED CELLS CONTAINING D HEMOGLOBIN.
M. A. Baig*, A. A. Ansari and H. V. Malling
NIH

P-13  A STUDY OF THE INDUCTION OF UNSCHEDULED DNA SYNTHESIS BY PHYSICAL AND CHEMICAL AGENTS IN NON-PROLIFERATING PRIMARY CULTURES OF RAT HEPATOCYTES.
D. A. Casciano and J. W. Oldham*
National Center for Toxicological Research

P-14  MUTAGEN-INDUCED CHROMOSOME LESIONS IN LYMPHOCYTES OF SAGUINUS OEDIPUS TAMARINS - A POSSIBLE GENETIC MARKER FOR ANIMALS AT RISK FOR COLON CANCER.
A. M. Sayer, L. G. Littlefield, R. J. DuPrain and C. B. Richter*
Oak Ridge Associated Universities

P-15  NONDESTRUCTIVE CYTOGENETIC TOXICOLOGIC TESTING WITH RATS.
P. A. Beltz* and R. D. Benz (Introduced by S. A. London)
University of California, Irvine - Dayton, OH
P-16  THE EFFECT OF AROMATIC AMINES ON SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS.
    IBM Corporation

P-17  SISTER CHROMATID EXCHANGES IN VIVO IN MICE: SYNERGISTIC INTERACTION BETWEEN TRIETHYLENEMELAMINE AND BROMODEOXYURIDINE.
    J. L. Wilmer and E. R. Soares
    Chemical Institute of Toxicology

P-18  CYTOGENETIC EFFECTS OF cis-PLATINUM(II)DIAMINEDICHLORIDE (cis-PDD) ON RABBIT AND HUMAN CELLS.
    W. D. Morrison, V. Huff, S. Colyer*, G. Littlefield and R. DuFrain
    Oak Ridge Associated Universities

P-19  INDUCTION OF SPECIFIC LOCUS MUTATIONS AND SIBLING ("SISTER") CHROMATID EXCHANGES BY BROMODEOXYURIDINE IN CHINESE HAMSTER OVARY CELLS.
    J. S. San Sebastian, J. P. O'Neill, A. Johnson* and A. W. Hsie
    University of Tennessee Oak Ridge Graduate School of Biomedical Sciences and Oak Ridge National Laboratory

P-20  SISTER CHROMATID EXCHANGE IN HUMAN FIBROBLAST-RAT HEPATOCYTE CO-CULTURES.
    A. D. Kligerman and G. Michalopoulos*
    Duke University

P-21  INDUCTION OF SISTER CHROMATID EXCHANGES BY CIGARETTE SMOKE CONDENSATE FRACTIONS WITH OR WITHOUT S-9.
    W. F. Benedict, J. A. Dawson* and A. Banerjee*
    Children's Hospital of Los Angeles and University of Medicine, Los Angeles

P-22  TENTATIVE IDENTIFICATION OF CHO-UV-1 AS A UV-SENSITIVE MUTANT DEFICIENT IN POST-REPLICATION REPAIR.
    L. L. Hinkle*, C. A. Waldren and T. D. Stamato*
    Eleanor Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center, and The Wistar Institute of Anatomy and Biology

P-23  POSTREPLICATION REPAIR IN YEAST.
    M. A. Resnick, J. Bryce and B. Cox (Introduced by J. Drake)
    N I E H S and Oxford University, England

P-24  GENETIC COMPLEMENTATION ANALYSIS OF MUTATIONS AFFECTING DNA REPAIR IN CHO CELLS.
    K. W. Brookman*, D. B. Busch*, L. H. Thompson, C. L. Mooney* and Donald A. Glaser*
    Lawrence Livermore Laboratory and University of California, Berkeley
P-25  SISTER CHROMATID EXCHANGE ANALYSES IN RODENT EMBRYO TISSUES AFTER IN VIVO BRDU SUBSTITUTION.
N I E H S

P-26  THE EFFECT OF PRIOR PARTIAL HEPATECTOMY OR CO-ADMINISTERED TOLUENE ON BENZENE-INDUCED BONE MARROW DAMAGE.
Brookhaven National Laboratory

P-27  THE EFFECT OF BIOTRANSFORMATION OF 2,4-DINITROTOLUENE ON ITS MUTAGENIC POTENTIAL.
D. B. Couch, D. J. Abernethy, P. F. Allen and D. L. Ragan*
Chemical Industry Institute of Toxicology

P-28  THE GENERATION OF PHENOTYPIC DIMORPHISM IN CELLS OF BLOOM'S SYNDROME: SOMATIC RECOMBINATION AS A MECHANISM.
A. B. Krepinsky* and J. A. Heddle
York University, Ontario

P-29  THE MICRONUCLEUS ASSAY: TESTING WITH AN IMPROVED PROTOCOL.
M. F. Salamone and J. A. Heddle
York University, Ontario

P-30  EVALUATION OF MUTAGENS FROM COOKED HAMBURGER WITH SEVERAL SHORT-TERM MAMMALIAN BIOASSAYS.
Lawrence Livermore Laboratory and University of California, Livermore and Berkeley

P-31  IMPROVED ISOLATION AND CHARACTERIZATION OF MUTAGENIC FRACTIONS FROM COOKED GROUND BEEF.
J. Felton, S. Healy*, D. Stuermer*, C. Berry*, H. Timourian*, F. Hatch, L. Bjeldanes and M. Morris*
Lawrence Livermore Laboratory and University of California, Livermore and Berkeley

P-32  A MODIFICATION OF THE AMES TEST WHICH ALLOWS FOR CALCULATION OF TRUE MUTANT FREQUENCIES.
E. J. Greene and M. A. Friedman
Allied Chemical Corporation

P-33  INCREASED MUTAGENICITY OF CARCINOGENIC NITROSO COMPOUNDS USING HAMSTER LIVER S9 PREPARATIONS.
R. Rainerl*, J. A. Poiley* and A. W. Andrews
Frederick Cancer Research Center

P-34  UV MUTATIONAL SPECIFICITY OF THE DRUG RESISTANCE PLASMID pKM101.
R. G. Fowler*, L. McGinty*, L. Komar* and K. Mortelmans
San Jose State University and SRI International
P-35 APPLICATION OF MUTAGENICITY TESTING TO DETECT MUTAGENS IN HUMAN URINE.
K. Mortelmans, G. Shan, K. Hawk-Prather* and J. H. Peters*
SRI International

P-36 NITRITAZOLE AS A MUTAGEN: ACTIVATION BY BACTERIAL BUT NOT MAMMALIAN NITROREDUCTASE.
L. W. Meyer*, J. L. Blumer* and W. T. Speck
Case Western Reserve University

P-37 GENETIC TOXICOLOGY OF SUBSTITUTIONALLY INERT TRANSITION METAL COMPLEXES.
G. R. Warren, P. N. Schultz*, E. H. Abbott* and S. J. Rogers
Montana State University

P-38 METHODS FOR THE DETECTION OF MUTAGENIC GASES AND VOLATILE LIQUIDS IN THE SALMONELLA/MICROSOME ASSAY.
J. F. Russell, Jr.*, M. E. Sippel* and D. F. Krahn
E. I. du Pont de Nemours & Company

P-39 QUANTITATIVE MEASUREMENT OF THE MUTAGENICITY OF VOLATILE LIQUIDS IN THE AMES SALMONELLA/MICROSOME ASSAY.
E. D. Barber, W. H. Donish* and K. R. Mueller*
Eastman Kodak Company

P-40 USE OF THE SPIRAL PLATER™ WITH THE SALMONELLA TEST FOR CHEMICAL MUTAGENS.
N. L. Couse* and J. W. King
University of Denver and Denver Research Institute

P-41 A PORTABLE FIELD UNIT FOR IN SITU USE OF THE AMES TEST.
P. R. Politte, M. E. Schrod and W. R. Lower
University of Missouri

P-42 MUTAGENICITY ASSESSMENT OF LIQUID EFFLUENTS FROM OIL SHALE TECHNOLOGIES.
B. J. Barnhart, S. H. Cox*, W. D. Spall* and R. T. Okinaka
University of California, Los Alamos Scientific Laboratory

P-43 A COMPARISON OF THE CAPACITY OF FROG AND RAT LIVER HOMOCENATES TO ACTIVATE PROMUTAGENS IN THE AMES TEST.
A. M. Cheh, A. B. Hooper*, C. Henke*, J. Skochdopole* and R. G. McKinell (Introduced by D. J. Picciano)
University of Minnesota, Gray Freshwater Biological Institute, Navarre and University of Minnesota, St. Paul

P-44 PREDICTING LONG TERM EFFECTS FROM SHORT-TERM TESTS DURING DRUG DEVELOPMENT.
R. S. Lake and F. A. de la Iglesia*
Warner-Lambert/Parke-Davis Pharmaceutical Research Division

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P-45 THE MUTAGENICITY OF SOME PROPOSED METABOLITES OF A BENZIDINE DERIVED DYE AND A PIGMENT IN THE SALMONELLA TYPHIMURIUM ASSAY SYSTEM.
National Center for Toxicological Research

P-46 COMPARATIVE MUTAGENIC ACTIVITY OF PARTICLE BOUND ORGANICS FROM COMBUSTION SOURCES.
J. L. Huisinigh and L. Claxton
US EPA, Research Triangle Park

P-47 MUTAGENIC AND LETHAL EFFECTS OF A SERIES OF m-DIAMINO-BENZENE DERIVATIVES IN SALMONELLA TYPHIMURIUM AND SACCHAROMYCES CEREVISIAE.
M. M. Shahin, A. Bugaut* and G. Kalopissis* L'Oreal Research Laboratories, France

P-48 FORMATION OF PURE AND MOSAIC MUTANT CLONES AND THEIR RELEVANCE IN CARCINOGENESIS.
M. A. Hannan and A. Nasim Ephraim McDowell Community Cancer Network, Lexington and NRC of Canada, Ottawa

P-49 APPLICATION OF A MODIFIED PROTOCOL TO SIMPLIFY THE SACCHAROMYCES CEREVISIAE D3 ASSAY.
G. Shepherd*, E. Riccio*, W. Tanaka, and K. Mortelmans SRI International

P-50 A SIMPLE, RAPID PLATE ASSAY FOR MITOTIC RECOMBINATION.
R. A. Kunz, B. J. Barclay and R. H. Haynes York University, Ontario

P-51 THE ACTIVATION OF PROMUTAGENS BY GREEN PLANTS.
J. M. Gentile and M. J. Plewa Hope College and University of Illinois

P-52 CYTOGENETIC TOXICOLOGIC TESTING WITH DOGS.
R. D. Benz and P. A. Beltz* University of California, Irvine – Dayton, OH

P-53 A METHOD FOR DETECTING REACTIVE ELECTROPHILES IN ENVIRONMENTAL SAMPLES.
A. M. Cheh and R. E. Carlson* Gray Freshwater Biological Institute, University of Minnesota

P-54 COVALENT BINDING OF CHEMICAL CARCINOGENS AND MUTAGENS TO RAT HEMOGLOBIN.
M. A. Pereira and L. Chang* US EPA, Cincinnati
POSTER PRESENTATIONS
continued

P-55  HUMAN 3-METHYLADENINE-DNA GLYCOSYLASE AS A PROBE FOR DETERMINING ALKYLLATION DAMAGE AND REPAIR IN HUMAN CELLS.
        T. P. Brent (Introduced by A. D. Welch)
        St. Jude Children's Research Hospital

P-56  NORHARMAN AND ELLIPTICINE: A COMPARISON OF THEIR ABILITIES TO INTERACT WITH DNA IN VITRO.
        J. Ashby*, B. Elliott and J. A. Styles
        Imperial Chemical Industries, Ltd., United Kingdom

P-57  DEVELOPMENT AND APPLICATIONS OF AN EQUILIBRIUM DOSE RESPONSE MODEL.
        D. J. Schaeffer, K. G. Janardan and H. W. Kerster
        Illinois EPA, Sangamon State University, Springfield, and
        California State University, Sacramento

P-58  GENETIC SEGREGATION IN MICE DETECTED BY TWO-DIMENSIONAL ELECTROPHORESIS.
        A. S. Raj and R. R. Marshall*
        York University, Ontario

P-59  OOCYTE KILLING IN MICE AS AN IN VIVO GAMETIC CYTOTOXICITY ASSAY: RESULTS FOR 45 CHEMICALS.
        Ryan*, T. C. Kwan* and R. L. Dobson
        Lawrence Livermore Laboratory, University of California

- NOTES -
abstracts

Aa-1

RELATIVE CONTRIBUTIONS OF B AND T LYMPHOCYTES IN THE HUMAN PERIPHERAL BLOOD MUTAGEN TEST SYSTEM AS DETERMINED BY CELL SURVIVAL, MITOGENIC STIMULATION AND INDUCTION OF CHROMOSOME ABERRATIONS BY RADIATION. Mary Esther Gaulden and Jeffrey L. Schwartz, University of Texas Health Science Center at Dallas.

Subpopulations of lymphocytes, separated on basis of differences in cell surface receptors, were studied to determine whether the frequency of chromosome aberrations might be influenced by a differential radiation response (50-500 rad γ rays) of three cell types: T, B and null lymphocytes. In unirradiated, PHA-stimulated 48 and 72 h cultures, 10-15% of the total mitotic cells are B lymphocytes; it was found that B cells are much slower than T cells in transforming, so that 50% of B cells are only in first division at 72 h. T, B and null cells have biphasic radiation survival curves (5 d), the $D_0$ for the sensitive portion being 60 rad for all three cell types; the $D_0$'s for the resistant portions were 270 rad for B, 477 rad for T and 603 rad for null cells. Mitosis is much more radiosensitive in B than in T or null cells. In 48 h cultures the proportions of mitotic B cells decrease with increasing doses of radiation to reach near zero after 250 rad. The frequencies of chromosome aberrations (acentric fragments and dicentrics + acentrics) were not significantly different in first division T and B cells; both 1-hit and 2-hit aberrations could best be fitted to linear-quadratic equations. The human whole blood culture is a complicated cell system for mutagen testing.

Aa-2

STATISTICAL ANALYSIS OF MUTAGENESIS TEST DATA: AMES/SALMONELLA, CHO/-HGPRT, MOUSE LYMPHOMA TK SYSTEMS, J. D. Irr and R. D. Snee*, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

Commonly used parametric statistical methods such as Student's t-test should only be applied to data sets satisfying certain conditions. Mutagenesis data gathered from three well established short-term tests, the Ames Salmonella/microsome assay, the CHO/HGPRT assay and the Mouse Lymphoma TK locus assay were evaluated to determine if they conform to these criteria. Mutations, being rare events, should be distributed in populations of cells or organisms according to the Poisson formula. A number of statistical models for mutagenesis data analysis have been formulated after making that assumption. We find that data sets from all three assays neither follow Poisson distribution nor satisfy assumptions needed to perform parametric tests. We chose to transform our data sets according to the power formula $Y = (X + A)^λ$ where $X$ is the observed mutation frequency, A and λ are constants and Y is the transformed response. A method was developed to choose A's and λ's yielding transformed values with homogeneous residual standard deviations and with experimental errors following a Gaussian distribution thus satisfying conditions for the application of parametric tests. For the Ames test, with any of the strains of bacteria, a λ of 0.2 and an A of 0 was satisfactory. The CHO and Mouse Lymphoma tests required a λ of 0.15. For CHO data, A was set equal to 1 but the Mouse Lymphoma test could be studied with A set to 0. After transforming a data set from a mutagenesis experiment, test doses are now compared to the negative control by a t-test of significance and for a dose response by analysis of variance techniques.
GENETIC EFFECTS OF FLAVONOLS IN CHINESE HAMSTER OVARY(CHO) CELLS IN VITRO, AND IN RABBIT LYMPHOCYTES AND MOUSE ERYTHROBLASTS IN VIVO. J.T. MacGregor, A.V. Carrano and J.H. Carver, USDA Western Regional Research Ctr., Berkeley, CA 94710 and Lawrence Livermore Laboratory, Livermore, CA 94550.

Forward mutation at four loci, cytogenetic alterations, and sister chromatid exchanges (SCEs) were measured in a single population of CHO cells exposed to quercetin (Q) for 15 hr. Q increased the frequency of isochromatid deletions per cell (e.g., 20% to 24%, 14% to 22% at 15 and 9 μg/ml vs 0% in controls) and chromosome exchanges (quetin 9% to 2% at 15 and 9 μg/ml vs 0.5% to 0.6% in controls). Significant, but smaller, increases in the frequencies of chromatid deletions and exchanges were also observed.

EMS, in contrast, produced mainly chromatid aberrations. An increased frequency of tetraploid cells was noted at 12 and 15 μg/ml, but not at 6 or 9 μg/ml. Cell survival was approx. 44% at 9 μg/ml and 22% at 15 μg/ml. Increased mutation frequencies were not observed at the HGPRT, APRT and Na+/K+ ATPase loci, but a small, significant, increase was observed at the TK locus (e.g., 2.24 ± 2.0x10^-3 FUDR resistant colonies per surviving cell at 9 μg/ml Q vs 1.0 ± 1x10^-3 in controls). The SCE frequency was slightly increased at some dose levels, but there was no clear dose response. Q, rhamnetin, and galangin (G) were tested at doses of 33 to 1000 mg/kg i.p., and Q at 1 g/kg p.o., in the mouse polychromatocyte micronucleus (MN) test at sampling times of 1-4 d. Small increases in the MN frequency were observed in one Q and two G test groups, but consistent increases were not observed. C was toxic at doses of 100 mg/kg and above. Mortality was increased by pretreatment with Arochlor-1254.

Rabbits given 1 g Q in a single dose, or in 2 or 4 divided doses 7 d apart, showed no increase in the frequencies of SCEs in peripheral lymphocytes at sampling times of 1, 7, or 14 days following each dose. (Performed under USDA contract 53-9AHZ-8-1443).
THE RELATIONSHIP BETWEEN EARLY DEATHS AND IMPLANTS IN CONTROL AND MUTAGEN TREATED CD-1 MICE IN DOMINANT LETHAL ASSAYS. Diana Anderson, D B McGregor and T M Weight†† ICI Ltd, Central Toxicology Laboratory, Alderley Park, UK †Inveresk Research International Edinburgh, UK.

In dominant lethal assays a premise is held that the number of early deaths is a property of the germ cells and not of the mated females. Thus, it has been assumed that there is a direct relationship between the numbers of early deaths and the number of total implants. This relationship is important in deciding the method of statistical analysis. The present communication investigated this relationship by examining the data from dominant lethal assays generated in two laboratories in the CD-1 mouse, both with or without mutagen treatment. The mutagens used were cyclophosphamide and ethyl methanesulphonate. Early deaths, and early deaths per implant as a function of implants were examined and the implications of the results generated have been considered. For negative control data when early deaths (E) were considered as a function of implants (I) it was found that a direct relationship between early deaths and implants only held at very low and very high implant numbers. Between 4 and 15 implants (based on over 7,000 females) the average number of early deaths remained constant. After treatment the direct relationship extended at low implant numbers over a larger implant range but again above this range up to 15 implants the average number of early deaths remained constant. It followed therefore that when early deaths per implant (E/I) as a function of implants were considered, the average values were dependent on I over the whole implant range, tending to decrease as I increased. The effect of the mutagens on E/I was greater at low implant numbers. Thus the present communication shows that the relationship between the numbers of early deaths and the numbers of implants may not just be a property of the germ cells in CD-1 mice.


Screening male progeny for translocation heterozygosity may be accomplished through fertility testing (sequential or conventional) with cytological analysis of steriles and partial steriles, or through direct cytological analysis of all progeny. The sequential procedure is the one used routinely in our laboratory. It involves individually mating test males with highly fertile females and scoring one or two litters. Using a litter size of 10 for classifying normal males we have estimated (through extensive fertility and progeny tests) that the error of misclassifying translocations as normals is < 5% (Generoso et al., Genetics 77:741-752, 1974). Because of the need to standardize the size of error of misclassification, we conducted a study comparing the sequential procedure as used in our laboratory with the cytological procedure (scoring 25 cells/mouse) as described by Adler (Biol. Zbl. 97:441-451, 1978). Coded male progeny from TMR-treated male parents were subjected to both procedures. Although the study is still in progress, complete information is already available for 117 mice. Of these, 49 mice were found to be translocations by both procedures, one male was detected cytologically but not through fertility, and one male showed partial sterility but was normal cytologically. One of the 49 males had only one cell out of 25 with multivalent association, and no evidence of translocation was found when 167 more cells were scored. On the whole, the efficiencies of the two procedures are highly comparable. Our early estimate of the misclassification error is consistent with the present result. [Research supported jointly by the Food and Drug Administration and the DOE under contract with UCC.]

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Aa-7
POINT (SINGLE GENE) AND VIABLE CHROMOSOMAL MUTATIONS IN L5178Y/TK+/-
CELLS CAN BE READILY DISTINGUISHED AND QUANTITATED. D. Clive, A.G.
Batson\*, K.O. Johnson\*, N. Turner\* and M.M. Moore-Brown\*, Wellcome
Two classes of thymidine kinase-deficient (TK/-) mutants arising from L5178Y/TK+
mouse lymphoma cells can be distinguished on the basis of colony size following incubation in trifluorothymidine-supplemented
soft agar cloning medium. Small colony TK/- (σ TK/-) mutants and large
colony TK/- (λ TK/-) mutants have been deduced to represent multi-locus
(chromosomal) mutations and single gene mutations, respectively, which
completely delete TK enzyme activity (Clive et al., Mutation Res. 59
(1979) 61-108). Colony size is heritable for both σ and λ mutants, and is
inversely proportional to doubling time in suspension culture; the
σ phenotype is spontaneously mutable at estimated frequencies of 10^-5.
λ TK/- mutants are usually induced linearly with dose or time, while
σ TK/- mutants typically arise with multi-hit kinetics. Mutagen
specificity exists: EMS and ICR-170 induce predominantly λ TK/- mutants
while MMS and hycanthone generate mostly σ TK/- mutants. Of these same
4 mutagens only EMS induces appreciable mutagenicity at the base pair
substitution-specific ouabain-resistance marker. These combined results
are consistent with the hypothesis that λ TK/- mutants represent single
gene mutations of frame shift or base pair substitution origin while
σ TK/- mutants represent more extensive damage involving the TK and
linked loci. This represents a unique capability to detect and distinguis
by eye alone both viable chromosomal and single gene damage in
eukaryotic cells and interprets some Ames-negative, σ TK/- positive
mutagens/carcinogens such as natulan and methyprilene in terms of a
mechanism of exclusively chromosomal mutagenicity.

Aa-5
MUTATION INDUCTION AT MULTIPLE GENE LOCII IN CHINESE HAMSTER OVARY CELLS:
COMPARING THE THYMIDINE KINASE LOCUS WITH THE AAL, TG, AND OUA MARKERS.
J.H. Carver, Lawrence Livermore Laboratory, Livermore, CA 94550 and G.M.
Agair, Science Park Research Division, University of Texas System Cancer
Center, Smithville, Texas 78957.
Mammalian genetic loci may differ markedly in their relative
mutability by a particular agent. We have selected a Chinese hamster
ovary cell line that is heterozygous for both the aprt and tk loci,
allowing single step selection of autosomal recessive AAL and FUDR
mutant phenotypes, as well as OUA and TG mutants. We have
compared mutational response of these loci to UV-irradiation, EMS, MMS,
M, ICR-191, DMN, and BaP. When compared after optimal expression
times, the four marker loci show mutagen-specific differences in their
relative mutability. Of the markers, the tk locus has consistently
shown the greatest mutational response to each of the mutagens tested.
Comparison of induced mutant frequencies at equivalent cell survival
(at the D50 dose) following mutagen exposure shows the following
order of relative potency—

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<thead>
<tr>
<th>Mutant</th>
<th>EMS &gt; ICR-191 &gt; DMN ≥ BaP ≥ UV &gt; MMS = MMC;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>EMS &gt; ICR-191 &gt; DMN ≥ UV &gt; BaP ≥ MMS &gt; MMC;</td>
</tr>
<tr>
<td>OUA</td>
<td>EMS &gt; ICR-191 &gt; DMN ≥ BaP ≥ MMS ≥ ICR-191 ≥ MMC;</td>
</tr>
<tr>
<td>FUDR</td>
<td>DMN = BaP &gt; EMS &gt; ICR-191 = MMS &gt; MMC &gt; UV.</td>
</tr>
</tbody>
</table>

Thus, mutagenesis assays employing multiple genetic markers may be more
effective than single locus assays in detecting a broad spectrum of
potential mutagens and carcinogens.
This work supported jointly by U.S. DOE contract W-7405-ENG-48; EPA
Interagency Agreement IAG-D5-E681-AN, AO; NIH 5 RO1 CA-04484.

47
COMPARISON OF MUTAGENIC, CONVERTOGENIC AND RECOMBINOGENIC EFFECTS OF SOME ADENINE ANALOGUES IN SACCHAROMYCES CEREVISIAE D7. W. G. Sorensen,* J. P. Simpson,* and T. Ong, intr. by J. A. Elliott, Microbiology Section, DRDS, NIOSH, 944 Chestnut Ridge Road, Morgantown, WV 26505.

Janion (1978, Mutation Res., 56:225) reported that 2-amino-N^6-hydroxyadenine (AHA) was the most potent mutagen of a series of base analogues studied in a Salmonella typhimurium spot-plate test system. More recently, Brockman, Hung, de Serres and Ong (1979, Environmental Mutageneisis 1:115) reported that AHA and 6-N-hydroxyaminopurine (HAP) are also potent mutagens at the ad-3 region in growing cultures of Neurospora crassa. We compared the activities of HAP and AHA with 2-amino purine (2AP) in growing and nongrowing cells of Saccharomyces cerevisiae D7 with regard to induction of gene conversion, mitotic recombination and reverse mutation. Ethyl methanesulfonate (EMS) was used as a positive control. No increases above spontaneous frequencies were observed when nongrowing cells were treated with the base analogues although EMS induced responses comparable to those reported by Zimmerman (1975, Mutation Res., 28:381). When growing cells were treated, HAP was convertogenic, recombinogenic and mutagenic; AHA was mutagenic but not convertogenic or recombinogenic at 200 \(\mu g/mL\); and 2AP failed to induce any detectable response even at 800 \(\mu g/mL\). HAP induced comparable numbers of revertants at much lower concentrations than AHA.


The oxidation of 4-ABP to N-OH-4-ABP by the cytochrome p450 enzymes of liver is the crucial step in the activation of this bacterial frameshift mutagen. In the present study a comparison was made between the capability of a given 9000 g supernatant fraction of liver to N-hydroxylate 4-ABP and to activate 4-ABP as a mutagen in S. typhimurium strain TA98. Correlation was excellent. Enzyme from various non-induced species activated 4-ABP as follows: hamster > guinea pig, mouse > rat. After Aroclor 1254 induction (100 mg/kg), enzyme from all four species were nearly equally effective. Variation of Aroclor dosage in rat resulted in maximum reversion at doses of 100 mg/kg or lower, and indicated that a dose of 500 mg/kg was superfluous. 3-Methylcholanthrene enhanced 4-ABP activation in both guinea pig and rat. Phenobarbital however induced only in guinea pig and not in rat. It was noted that the number of revertants increased sharply with increasing enzyme concentration up to levels of at least the equivalent of 25 mg liver wet wt. The study illustrates that the apparent potency of a promutagen can be greatly influenced by choice of activation system with respect to species, inducer, inducer dosage, enzyme levels used, etc.
AA-11

SIMULTANEOUS DETERMINATION OF HISTIDINE REVERTANTS AND 8-AZAGUANINE MUTANTS IN A SINGLE CULTURE OF S. TYPHIMURIUM STRAIN TA-100. J.L. Seed and E. Bueding, Johns Hopkins School of Hyg. & Public Health, Baltimore, MD 21205

A sensitive method has been developed for the simultaneous determination of point mutations (Histidine reversions) and gene mutations (8-azaguanine resistance) (8-AGR) in Salmonella Typhimurium strain TA-100. The procedure is a modification of a previously described method (Skopek et al, Proc. Natl. Acad. Sc. 75:410-412, 1978). In this modification, the conditions of exposure to mutagens have been altered to include a 1 hour exposure in a buffered salt solution followed by a 3 hour exposure in a nutrient-supplemented salt solution. Furthermore, the conditions of culture growth have been altered to reduce the amount of nutrients in the culture medium. As a result, the spontaneous mutation rate and variability of the assay have decreased while the response to chemically induced mutations has increased. The spontaneous point mutation (Histidine reversion) rate 6.8 mutants/10⁴ colony forming units (CFU) and the spontaneous gene mutation (8-AGR) rate was 2.5 mutants/10⁵ CFU. Mutagens such as 3-chloromethylpyridine (250 µg/ml), paraziquantel (150 µg/ml) and lead subacetate (250 µg/ml) were all positive in the gene mutation system (8-AGR) with induced mutation rates of 13.56/10⁶ CFU, 5.89/10⁵ CFU and 14.19/10⁵ CFU respectively. The test system was also amenable to body fluid analysis. 8-Glucuronidase treated urines of animals receiving 200 µg/ml of the carcinogens 2-methyl-4-dimethlaminoazobenzene and 4-dimethylaminoazobenzene gave mutation rates of 6.4/10⁵ CFU and 5.5/10⁵ CFU compared with a control mutation rate of 2.3/10⁵ CFU in this system. The histidine reversion system was slightly less sensitive. Results indicate that a forward mutation assay to 8-Azaguanine resistance is a useful alternative to the widely used histidine reversion plate assay.

AA-12

THE APPARENT NON-MICROSOMAL ACTIVATION OF 2-ACETYLMAMINOFLUORENE TO A MUTAGEN IN VITRO. D.B. McGregor, Inveresk Research International Limited, Edinburgh, EH21 7UB, Scotland.

2-Acetylaminofluorene (AAF) which appears not to be carcinogenic in cotton rats or guinea pigs, but is highly carcinogenic in rats, can be activated to a mutagen for Salmonella typhimurium by post-mitochondrial supernatant fractions from both rats and cotton rats. The activation reactions are thought to involve microsomal N-hydroxylation followed by deacetylation and/or esterification to an unstable proximate mutagen which ionizes to the ultimate mutagen which reacts with DNA. Further fractionation procedures have revealed that twice-washed microsomes can mediate in the activation process in vitro, in the presence of a NADPH-generating system. Activation can also be achieved with cotton rat (but not rat) microsomes without NADPH and with cotton rat and rat microsome-free cell sap. Characteristics of these rat and cotton rat systems and the metabolites generated in them will be compared. The involvement of bacterial metabolism in the Salmonella/activation system will also be discussed.

<table>
<thead>
<tr>
<th>S. typhimurium TA 1538 Mutants with 1 µg AAF</th>
<th>Cotton Rat</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-9</td>
<td>1,400</td>
<td>45</td>
</tr>
<tr>
<td>Microsomes w/o NADPH</td>
<td>410</td>
<td>19</td>
</tr>
<tr>
<td>Cell sap</td>
<td>280</td>
<td>45</td>
</tr>
<tr>
<td>Microsomes + rat cell sap</td>
<td>1,000</td>
<td>-</td>
</tr>
<tr>
<td>Microsomes + cotton rat cell sap</td>
<td>-</td>
<td>310</td>
</tr>
<tr>
<td>Control</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

Renewed interest in the relationship between diet and cancer has stimulated a growing number of reports of mutagens or potential carcinogens in foods, including natural constituents, contaminants, auto-oxidation, nitrosation and pyrolysis products. To put these reports into perspective we have undertaken a general survey of foods, starting with beverages, for the presence of mutagenic activity using the Salmonella assay. Widely consumed products from 13 beverage categories were selected randomly. Each sample was concentrated and fractionated by polarity and solubility to give 7 fractions. Each fraction was assayed for mutagenicity with Salmonella TA98 and TA100 ± S9 and the results interpreted according to predefined criteria. To establish sensitivity of the procedure, different beverages were spiked with known mutagens prior to fractionation. Of the 28 different beverages only a few were found to contain slight mutagenic activity in this screening survey.

PURIFICATION AND CHARACTERIZATION OF MUTAGENS FORMED FROM THE REACTION OF SPERMIDINE WITH NITRITE. H. Kong and M.L. Murray, Department of Microbiol and Immunology, LSU Med Ctr, New Orleans, La. 70119

It has been reported that the natural polyamine spermidine reacts with nitrite at pH 3-5 to produce direct-acting mutagenic activity. Initial fractionation of the reaction mixture (0.06M spermidine, 0.3M nitrite, pH 4.2) revealed at least 8 distinct products by thin layer chromatography (TLC). Two fluorescence-quenching bands observed on TLC were demonstrated to be associated with mutagenic action, using Salmonella typhimurium tester strain TA1950. Absorption spectra of TLC purified products showed a prominent peak at 240 nm associated with the two bands containing mutagenic activity. These mutagenic products account for not more than 6% of the spermidine added to the reaction mixture. With radioisotope labeled spermidine in the reaction, specific activities of the two mutagenic bands were estimated to be about 3.6 and 2 revertants per nanomole, respectively. This degree of mutagenicity is comparable to direct-acting mutagenic nitrosamines such as N-nitrosomethylurea, and is about 20-fold more potent than most nitrosamines after enzyme activation.

INDUCTION OF REVERSE MUTATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS. J. C. Fusco, J. P. O'Neill, and A. W. Hsie. The Univ. of Tenn.-Oak Ridge Graduate School of Biomedical Sciences and the Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Conditions have been defined for quantifying reversion of 6-thioguanine resistant CHO cells using the heterocyclic nitrogen half-mustard ICR-191. In reconstruction experiments, where 100 hypoxanthine-guanine phosphoribosyl transferase-positive (HGPRT+) cells were co-cultured with an increasing number of HGPRT- cells, we found that recovery of HGPRT+ cells was complete to a density of $2 \times 10^6$ HGPRT- cells/100 mm dish in medium F12 containing $10^{-5}$ M azaserine. Employing the CHO/HGPRT forward mutation assay, cells were isolated and found to have no detectable HGPRT activity. They were then treated with ICR-191 and plated in the azaserine medium on
various days after treatment to determine the time necessary for expressing the revertant phenotype. It was found to be 1-3 days in most cases. Seven revertant clones were isolated and all were found to retain a stable phenotype in the absence of azaserine and to have HGPRT activity. Depending on the ICR-191-induced HGPRT- clone, the ICR-191-induced reversion frequency varied from $10^{-7}$ to $10^{-5}$ with a spontaneous reversion frequency of $10^{-7}$ revertants/cell. The finding that ICR-191-induced 6-thioguanine resistant clones having no HGPRT activity are revertible by ICR-191 is consistent with the notion that ICR-191 induces gene mutations in mammalian cells. It also provides further evidence that the CHO/HGPRT system detects structural gene mutations. (Research sponsored by the Office of Health and Environmental Research, U.S. Dept. of Energy, under contract W-7405-Eng-26 with the UCC. JCF is a predoctoral fellow supported by Grant CA 09104 from the NCI).

Ab-3

MUTAGEN INDUCED REVERSION OF A CHINESE HAMSTER OVARY TRIPLE AUXOTROPH.

R.T. Taylor and R. Wu.* Biomedical Sciences Division, Lawrence Livermore Laboratory, Livermore, CA, 94550.

A Chinese hamster ovary auxotroph (CHO AUXBl) exhibits growth require-
ments for glycine, adenosine, and thymidine (GAT) due to a defect in the
structural gene for folypolyglutamate synthetase (<3% parental FPGS)
(1979)). We have begun to examine quantitatively its reversion in re-
sponse to various mutagens. Following 20 h exposures in complete medium
(+GAT), maximal detection of revertants requires no or, at most, 48 h of
post-treatment expression growth. The cloning efficiencies of protrophic
revertants in deficient medium (-GAT) is not influenced by the presence
of large numbers of CHO AUXBl. Revertant selections are made by plating
1 x 10^6 cells/100 mm dish and incubating for 12 days in deficient me-
dium. Dose-dependent increases above the spontaneous AUXBl revertant fre-
quency (avg. 5 x 10^{-7}) were induced with cis-Pt(NH3)2Cl2 (14 X)
(but not trans-Pt(NH3)2Cl2), PtCl4 (10 X), Pt(SO4)2 (14 X),
K2CrO4 (8 X), EMS (10 X), 4-NQO (53 X), ICR-191 (60 X), ICR-170 (30
X) and glyoxal (50 X). Among 42 AUXBl revertants isolated thus far, all
are stable and are characterized by an increased growth incorporation of
[G-3H]folic acid. Extracts from 12/16 spontaneous, 6/6 Pt(SO4)2
induced, 4/6 4-NQO induced, 7/7 K2CrO4 induced, and 7/7 ICR-191
induced revertants contain 5-94% as much FPGS as the original parental
CHO cells. The data indicate that CHO AUXBl has utility as a supplemental
mammalian system for assessing the mutagenicity of metal complexes. More-
ever, AUXBl appears to be most susceptible to reversion by compounds that
are known to react with guanine bases in DNA. (Supported by U.S. DOE

Ab-4

MITOCHONDRIAL DNA SYNTHESIS IN HUMAN LYMPHOCYTES AFTER CONSUMPTION OF
NATURALLY OCCURRING NITRATE, NITRITE AND NITROSAMINES. C.T. Miller,
N.P. Sen and E. Lok. Foods Directorate, Health Protection Branch,
Ottawa, Canada.

An assay for genetic interaction in vivo was developed based on in vitro results indicating increased mtDNA synthesis after alklation, and preferential alklation of mtDNA relative to nuclear DNA by nitroso compounds. Volunteers donated saliva and blood samples before and 20, 40, 80 and 160 minutes after consuming meals of varying nitrate, nitrite, amine and nitrosamine content. Salivary nitrate and nitrite reached maxima proportional to dietary intake of vegetable nitrate. Volatile nitrosamines were occasionally detected in blood, and although
low, estimated total amounts exceeded those ingested, indicating in vivo formation. In most cases mtDNA synthesis in lymphocytes increased significantly post-meal. The magnitude of the increase was not proportional to salivary nitrite or blood nitrosamine level.

Ab-5
DETECTION OF N-METHYL-N'-NITRO-N-NITROSOGUANIDINE (MNNG) INDUCED INTESTINAL METAPLASIA IN THE GLANDULAR STOMACH OF RATS. Robin W. Morgan, Jerrold M. Ward* and Philip E. Hartman, The Johns Hopkins University, Baltimore, MD 21218 and National Cancer Institute (NCI), Tumor Pathology Branch, Bethesda, MD 20014.

Intestinal metaplasia (IM) can be induced in the glandular stomach of rodents by direct acting chemical carcinogens. IM foci generally are considered benign,preneoplastic lesions and functionally resemble small intestinal tissue. Intestinal alkaline phosphatase activity persists after exposure to formalin at room temperature; thus, its activity can be used to localize plaques of IM in whole stomachs preserved even for extended periods of time. Using tissue obtained from the NCI Tissue Repository, IM foci were scored in stomachs of male F344 rats given MNNG orally in an experiment terminated over 5 years ago (PHS Contract NOI-CP 712166). The results show an increase in the incidence of IM foci in MNNG treated animals. Alkaline phosphatase positive foci were found in: 1/9 control, 9/19 low dose (20 ppm), 6/8 medium dose (40 ppm), and 4/5 high dose (80 ppm) animals. Multiple IM foci were found in some treated animals. Our estimates of IM foci are probably low because not all portions of stomachs were available, and the glandular stomachs of many animals treated with MNNG were almost entirely occupied by tumors and therefore had little scorable tissue.

IM foci may share initiating mechanisms with tumor induction. Therefore simple tests for IM may be useful in the estimation of hazards posed by chemical agents, including weak carcinogens that yield equivocal results with conventional procedures.

Supported in part by 1 R01 CA26328-01, NCI, to PEH.

Ab-6

During fertilization the proteolytic enzyme acrosin appears to be involved in the penetration of the sperm through the zona pellucida. The acrosin activity of human sperm can be detected and measured in vitro (D. Propping, et al., Int. J. Fertil., 23:45-50, 1978). We tested whether or not acrosin activity can be altered in mice by the known mutagen Mitomycin C. C57B1 male mice were treated with 5 consecutive daily injections of 0.0, 0.5, 1.0, 1.5, and 2.0 mg Mitomycin C/kg b.w. Five weeks after the last injection, vas deferens sperm were recovered. The sperm were capacitated, spread on gelatin coated slides, and were incubated for 1 hr. @ 37°C. Acrosin activity was indicated by a halo of degraded protein around individual sperm heads which remained clear upon subsequent staining with toluidine blue. Sperm without acrosin activity lacked an unstained halo. The percentage of sperm with acrosin activity at the different doses was as follows (mg/kg b.w. Mitomycin C dose in parenthesis): 93.35±1.08 (0.0), 88.19±3.38 (0.5), 47.46±11.81 (1.0), 27.2±11.18 (1.5), 23.51±13.84 (2.0). In the same animals the percent of motile sperm was 60.34±2.27, 50.12±2.46, 8.23±5.61, 1.58±0.71, 8.91±5.68, respectively. The authors feel that study of sperm phenotypes such as acrosin activity and motility, along with sperm head abnormalities, are potentially useful methods for detection of environmental mutagens, carcinogens and teratogens.
Ab-7

Flow cytometric fluorescence measurements on DNA stained sperm from mice exposed to X-rays or methyl methane sulfonate (MMS) show dose dependent changes in the coefficient of variation (CV) of the fluorescence distributions. If the acriflavine-Fuigens fluorescence is proportional to DNA, these results demonstrate induced variability in DNA content of the sperm. We measured caudal epididymal sperm from: (a) B6C3F1 and AKD2F1 mice collected 14, 21, 35 and 70 days after testicular X-irradiation of 600 ra; (b) B6C3F1 and AKD2F1 mice collected 35 days after testicular X-ray exposure at 7 doses between 0 and 900 ra; and (c) B6C3F1 mice collected 35 days after the start of 5 daily, intraperitoneal injections of 4 doses of MMS. Three independent measurements were done at most dose levels on pooled samples from at least three mice. In the kinetic experiment (a), maximum increase in the CV occurred 35 days post irradiation, with slight response at 21 days and no response at 14 and 70 days. This indicates premeiotic damage. In the radiation dose response experiment (b), the CV increased from about 3% (0 ra) to a maximum of approximately 6% in the 450 to 600 ra range, and then decreased at 900 ra. Following MMS exposure, (c), the CV increased sharply as the dose approached the lethal level. The X-ray data show large pool-to-pool variability at each dose. This may reflect variations in biologic response because it is strongly correlated (r = 0.51; p < .01) with visual determinations of the fraction of abnormally shaped sperm per pool. (Supported by U.S. DOE contract W-7405-ENG-48, DOE/NIEHS Interagency Agreement No. 2ZZ-701-ES-7003 and DOE/EPA Transfer Agreement No. 79-y-7053).

Ab-8

Semen was collected from 50 men occupationally exposed to carbaryl (l-napthyl methyl carbamate) for durations of 1 to 18 years and compared to semen from a control group of 34 unexposed, new-hires. The exposed workers showed a significantly higher proportion of sperm with abnormal head shapes than did the control group (p < 0.005). This finding appears to be limited to men working in the carbaryl production area at the time of sampling. Formerly exposed workers did not show a statistically significant elevation in percent of abnormal sperm. Age, smoking habits, and medical problems did not appear to affect these results. However, the increase in sperm abnormalities was not related to exposure dose (estimated by number of years on the job or job classification during the past year). Unexplainably, the increases in sperm abnormalities were seen mainly in those currently exposed men who had worked with carbaryl for less than 5 years. These findings demand further human and animal studies because the sperm abnormalities seen in the carbaryl workers may be due to unknown factors, which are always present in occupational settings. Whatever agent(s) induced the sperm abnormalities seen in the carbaryl workers, its effect was small since statistically less-sensitive assays (sperm counts and frequency of sperm with 2 fluorescent bodies) did not show a response. Work performed under the auspices of the U.S. DOE under contract number W-7405-ENG-48 and the U.S.EPA Pass-Through Agreement.
Ac-1


Coal-derived petroleum substitutes (syncrudes) exhibit greater mutagenicity than conventional petroleum-derived oil when assayed with the Salmonella/microsomal activation test. Alkaline constituents were found to be uniquely important in syncrude mutagenicity. Polycyclic aromatic primary amines appear to be more important in these isolates when compared with basic nitrogen heterocyclics. Parallel assays with a yeast system (histidine reversion, canavanine resistance) have reinforced the bacterial results with the fractionated basic materials. Direct acting components are observed. With neutral fractions both simple and highly alkylated polycyclic aromatic hydrocarbons contribute to the mutagenicity with a generally increasing biological activity with increasing ring number. Positive comparative results with model compounds aza-benzo(a) pyrene, 2-aminonaphthylamine, 1-aminonaphthacene, 1-aminopyrene, 3-aminopyrene, and 3-aminoperylene are illustrated. Research jointly sponsored by the Environmental Protection Agency (IAG-D5-E681) and the Office of Health and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

Ac-2

CHARACTERIZATION OF THE MUTAGENS ASSOCIATED WITH DIESEL PARTICLE EMISSIONS. Larry Claxton and Joellen Huisingh, Genetic Toxicology Division, Environmental Protection Agency, Research Triangle Park, NC 27711. The purpose of this study was to demonstrate means by which microbial and chemical methods could be combined to characterize the nature of the mutagens associated with Diesel particles. Light duty Diesel automobiles were run on an engine dynamometer and the particulate exhaust was collected on Pallflex glass-fiber filters. The organics were extracted from the particles with dichloromethane and solvent exchanged to dimethylsulfoxide. These organics were tested in the plate incorporation test with a nitroreductase deficient strain (TA98FRI) of Salmonella typhimurium as well as the regularly used strains TA98, TA1538, TA1537, TA1535, and TA100. Diesel organics, chemically fractionated into acidic, basic, nonpolar neutral, polar neutral and polymolecular aromatic hydrocarbon fractions were tested using the various bacterial strains. By comparing the results of TA98 and TA98FRI it was demonstrated that a portion of the mutagenic activity within Diesel organics is due to nitroaromatic compounds. For example, with organics from a Nissan automobile the response of TA98FRI is approximately one-half of the response of TA98 at each dose tested on the linear portion of the dose response curve. The most mutagenic fractions of the Diesel were the polar neutral components which showed direct acting mutagenic activity. TA1535 was generally negative with all types of samples. In summary, this work demonstrates that most of the mutagenic activity associated with organics from light duty Diesel automobiles is due to direct acting frame-shift mutagens and that a significant portion of the activity is due to nitrated compounds.
Ac-3

POSSIBLE MUTAGENIC/CARCINOGENIC ACTIVITY OF DIESEL PARTICULATES,
Mitchell Dukovich*, Ronald E. Yasbin, Samuel S. Lestz*, Terence H.
Risby and Roy B. Zweidinger*, The Pennsylvania State University,
University Park, PA 16802

In recent studies involving the Ames Salmonella mutagenicity test, and
the B. subtilis COMP Test, we have demonstrated that the total organic
fraction of diesel particulate has a mutagenic/carcinogenic potential.
Raw diesel particulate samples were generated from five different engines
operated under varying conditions and the total organic fraction was ex-
tracted. Increasing concentrations of this fraction was subjected to the
bacterial tester systems for determining mutagenic and carcinogenic poten-
tial. For each fraction tested in the Ames assay, an increase in concen-
tration of sample was followed by a subsequent increase in the number of
revertants (colonies) to histidine prototrophy. The number of revertants
ranged from one revertant per µg of sample to 25 revertants per µg of sam-
ple for strains TA98 and TA100. In addition, the toxicity of the organic
fraction on the Salmonella strains was determined. After one hour of in-
cubation at 37°C, 500 µg/ml of the extracts were lethal to no greater than
90 percent of the cells. In testing for the ability of the organic frac-
tion to induce bacterial SOS functions, competent cultures of B. subtilis
RUB827 were exposed to selected quantities of sample and the relative
transformation determined. The concentration of sample capable of reduc-
ing the relative transformation (RT) to 0.05, ranged from 100 µg/ml to
150 µg/ml. The toxicity of the samples for B. subtilis were also deter-
mained. Similar to the results with the Salmonella strains, 500 µg/ml of
the extracts were lethal to no greater than 90 percent of the Bacilli.
The data substantiates the concern over high levels of diesel particulate
pollutants in the environment.

Ac-4

COMMTAGENICITY OF BISULFITE. T.G. Rossman, R.G. Mallon*, New York Univer-
sity Institute of Environmental Medicine, New York, NY 10016.

Bisulfite, the physiological form of sulfur dioxide, is a major air
pollutant and a food additive. Human exposure to bisulfite due to these
sources is widespread. Epidemiological studies have linked atmospheric
SO₂ to many types of respiratory disease, including lung cancer. Inhaled
SO₂ has been shown to enhance the tumorigenicity of benzo(a)pyrene in
rats. Bisulfite is mutagenic to E. coli (Makai et al. BBRC 39:983, 1970)
at high concentration (1.0M) and pH 5.2. Its mutagenicity to animal
cells has not been demonstrated. We therefore decided to investigate the
mutagenicity of bisulfite to prokaryotic (E. coli) and eukaryotic
(Chinese hamster V79) cells under more moderate conditions. At non-toxic
doses, bisulfite does not induce trp⁺ revertants in E. coli, nor does it
cause mutations to ouabain-resistance in V79 cells. However, simulta-
naneous exposure to UV-irradiation and bisulfite, or exposure to bisulfite
immediately after UV-irradiation, enhances the UV-induced mutagenesis in
both systems. Trp⁺ revertants in E. coli WP2 were increased 6 fold by
simultaneous exposure, and 3.5 fold by incubation in bisulfite after UV.
Ouabain-resistant mutants were increased about 2 fold by either treat-
ment. Bisulfite has been shown to cause deamination of cytosine to uracil
and, at low concentration, DNA chain breaks via free radicals (Shapiro,
occurs under conditions of our experiments, the damage to DNA is not
sufficient to lead to mutagenesis. However, if the bisulfite-induced DNA
damage occurs in association with damage from another agent (UV), a syner-
gistic effect is seen. Alternatively, bisulfite itself may inhibit DNA
repair of pyrimidine dimers by another mechanism, resulting in enhanced
mutagenesis by UV.
MUTAGENICITY AND CYTOTOXICITY OF HALOETHANES IN THE CHO/HGPRT SYSTEM.
E. L. Tan* and A. W. Hsie, The Univ. of Tenn.-Oak Ridge Grad. Sch. of

The widespread use of haloalkanes as additives in industrial and house-
hold products poses an environmental concern. Here we report the cyto-
toxic and mutagenic activity of ethylene dibromide (EtBr2), ethylene di-
chloride (EtCl2) and ethylene chlorobromide (EtClBr) as determined in the
Chinese hamster ovary cell/hypoxanthine guanine phosphoribosyl transferase
(CHO/HGPRT) system. The cytotoxicity and mutagenicity were quantified by
determining the effects of each chemical on the cellular cloning ef-
ficiency and the frequency of mutation induction to 6-thioguanine re-
stance. We found that these ethyl halides are direct-acting agents with
EtBr2 being more cytotoxic and mutagenic than EtCl2. The mixed halogen-
ated congenor EtClBr has an intermediate effect. On a molar basis, the
relative mutagenic activity based on mutation frequency is approximately,
EtBr2: EtClBr:EtCl2 = 100:6:1 while cell survival is reduced to 50% sur-
vival by EtBr2, EtClBr, EtCl2 at approximately 3, 8 and 20 mM respectively.
Employing EtBr2, we observed an increase in mutation induction with an
increasing treatment time up to 24 hr. When these three compounds were
assayed in the presence of an Arochlor 1254 induced rat liver metabolic
activation system, S9, there was a 5-10 fold increase in cytotoxicity
without affecting the mutagenicity. This increase in cytotoxicity was not
observed when we omitted NADPH in the S9 system. Thus, EtBr2, EtCl2 and
EtClBr are direct-acting mutagens; however, NADPH is required in the S9
mix to exert further cytotoxicity. (Research sponsored jointly by EPA
under Interagency Agreement D8-E681-A0 and by DOE under contract W-7405-
eng-26 with the Union Carbide Corp.; ELT is a Postdoctoral Investigator.)

MUTAGENIC MYCOTOXINS FROM FUSARIUM MONILIFORME. L. F. Bjeldanes, L. A.
Weib*, Nutritional Sciences, University of California, Berkeley, CA 94720

Studies of mutagenic activity, as measured by the Ames assay, of ex-
tracts from many species of fungi have indicated that, although only a
small percentage of the species examined exhibited this activity, extracts
from 21 of 33 (64%) isolates of Fusarium moniliforme were active against
Salmonella typhimurium strain TA100. Of the 22 isolates of F. moniliforme
assayed against strain TA98 relatively weak mutagenic activities were
observed in 7 (33%). Results of studies of growth conditions for
optimum mutagen production by one isolate of F. moniliforme indicate
that growth at 23°C for 2 weeks followed by cold treatment (11°C) for 1
or 2 weeks produces up to a 4-to-5 fold increase in activity over back-
ground, compared to activity from ambient-temperature incubations. Toxi-
city of rice and corn contaminated with F. moniliforme was assayed in
day-old cockerels. No decrease in growth rate nor obvious abnormalities
appeared in birds fed for 21 days on diets containing 56% heavily
contaminated rice or corn. Several new mutagens were isolated from a
strain of F. moniliforme, and named fusariogenin A, B, C and D. Fusari-
genin C, the mutagen produced in largest quantity is a noncrystalline
compound with molecular formula C23H29O7. The ultraviolet, infrared,
and proton and carbon magnetic resonance spectra were recorded. Further
studies of the chemistry and biological activity of the fusariogenins
and other mutagenic metabolites of F. moniliforme are in progress.
Ac-7
MUTAGENICITY IN SALMONELLA OF DYES USED BY DEFENCE PERSONNEL FOR THE DETECTION OF LIQUID CHEMICAL WARFARE AGENTS. Earle R. Nestmann, David J. Kowbela, George R. Douglas and J.A. Wheat*, Mutagenesis Section, Environmental and Occupational Toxicology Division, Health Protection Branch, and Protective Sciences Division, Defence Research Establishment, Ottawa, Ontario, K1A 0L2, Canada.

Paper strips containing indicator dyes have been developed to change color upon exposure to certain agents but not to common solvents. Defence personnel and industrial workers who process the dyes and make the paper, may be at risk of hereditary disease or cancer if these dyes are mutagenic. As the preliminary step in our studies on their possible genetic hazard, several dyes in these detection papers were tested with the Salmonella/mammalian microsome assay, and 3 were found to be mutagenic. Orasol Navy Blue 2RB was mutagenic in 5 strains with the maximal increases found in strains TA1537, TA1538 and TA98, without S9 (44- to 96-fold increases). Eastman Fast Blue G-BLF was mutagenic in all strains but TA1535, the highest yields, enhanced by S9, again found in the frame-shift revertible strains (6- to 11-fold). Ethyl-bis (2,4-dinitrophenyl) acetate induced mutations only in strains TA1537, TA1538, and TA98, the greatest yield found in TA1538 without S9 (60-fold). 2,5,2',5'-tetramethyltr-phenylethene-4,4'-diao-bis-hydromaphthoic anilide and thiodiphenyl-4,4'-diao-bis-saliclyc acid were not mutagenic. Although microbial test results cannot be used to assess risk in man, these results reveal a potential hazard for individuals exposed to these dyes. Further work is underway using mammalian test systems.

Ac-8
MUTAGENICITY TESTING WITH L5178Y MOUSE LYMPHOMA CELLS, Andrea M. Rogers, Toxicology Branch, Air Force Aerospace Medical Research Laboratory, WPAFB, OH 45433.

Heritable effects can be detected in mammalian cells in culture using changes in gene function as an end-point. The function of different genes can be revealed by the use of selective agents. The four selective systems used here are ouabain, thymidine, thioguanine and cytosine arabinoside. Previous work with a series of eight different compounds in L5178Y mouse lymphoma cells has indicated that they may possess mutagen specific activity. Monomethylhydrazine (MMH), 1,1-dimethylhydrazine (UDMH) and 1,2-dimethylhydrazine (SDMH) have been examined for mutagenic potential and work is in progress with hydrazine. MMH did not induce a significant increase in mutation frequency in any of the four selective systems either with or without S9 activation. A range of doses of MMH was used giving a cell survival of 10-100%. UDMH and SDMH gave a significant increase in induced mutation in the thymidine selective system only in the absence of extraneous metabolic activation. A dose-response curve has been constructed for mutation induction in the thymidine system for both these compounds. The range in induced mutation frequencies per survivor was 1.35x10^-5 for 0.1 mM UDMH and 4.02x10^-5 for 5 mM UDMH. The highest induced mutation frequency per survivor for SDMH was 8.36x10^-5 for 5 mM SDMH. Currently, the results for two other compounds, a frame-shift mutagen, quinacrine mustard and a DNA intercalating agent, acridine orange are being studied. It is anticipated that the data from these compounds and that obtained from the hydrazines can be used to construct a comparative model indicating the activity of each selective system and the ability to determine different types of DNA damage.
Mutagenicity of Trenimon and 2,3,5,6-Tetraethylhyleneimino-1,4-Benzquinone (TEB) at the ad-3 Region of Neurospora Crassa. H. E. Brockman, C. Y. Hung, T. M. Ong, and F. J. de Serres, Illinois State University, Normal, IL 61761, and National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

2,3,5-triethyleneimino-1,4-benzquinone (Trenimon) and TEB are tri and tetrafunctional alkylating agents. Although the mutagenicity of Trenimon has been studied extensively, the mutagenic activities and specificities of these two chemicals have not been compared in many test systems. In Neurospora crassa, TEB reverts a presumptive base-pair substitution ad-3 mutant and a presumptive frameshift ad-3 mutant (Ong, 1978, Mutat. Res. 53:297), but there is no report on Trenimon to our knowledge. We are studying the mutagenicity of these two chemicals in heterokaryon-12 (H-12) of N. crassa, in which ad-3 mutants that are intracistronic lesions (ad-3R) or multicistronic deletions (ad-3IR) are recovered. In this strain, Trenimon has greater killing and mutagenic activities than TEB. Approximately 250 ad-3 mutants from each of three doses of TEB, which caused \( \nu \) 70, 300, and 900-fold increases over the spontaneous frequency, have been studied. The frequencies of ad-3R and of ad-3IR mutants per \( 10^6 \) survivors increased in proportion to the first and second power, respectively, of the dose of TEB. At the highest dose, \( \nu \) 11% of the ad-3 mutants are ad-3IR. We have also studied the mutagenicity of TEB in heterokaryon-59 (H-59), which is identical to H-12 except that it is homokaryotic for the excision repair-deficient gene \( uvs-2 \). Dose-response curves show that H-59 is much more sensitive than H-12 to the killing and mutagenic activities of TEB. We conclude that Trenimon is more mutagenic than TEB in H-12, that TEB induces ad-3R and ad-3IR mutants, and that TEB has greater killing and mutagenic activities in H-59 than in H-12. (Research supported by NIH.)

Ac-10


Gasification of low sulfur coals is being developed for an energy alternative as a part of this country's synthetic fuels program. Commercial gasification plants will produce large quantities of wastes, whose mutagenic and cytotoxic potential are discussed. We present further evidence that chemical fractions of volatile mutagenic gasifier crude tars exhibit considerable mutagenicity, as well as cytotoxicity to Chinese Hamster Ovary cells in culture. A raw coal semi-batch fixed bed gasifier operating at about 1000°F generated effluent samples. Four coals (Illinois #6, North Dakota lignite, Wyoming sub-bituminous, and Western Kentucky bituminous) and the crude tars produced by gasification were examined for mutagenic potential employing the Ames/Salmonella reverse mutation bioassay with bacterial tester strains TA98 and TA100. Crude tar effluents were subjected to a solvent partitioning scheme which generated six chemical groups: organic acids, organic bases, polynuclear aromatics, polar neutrals and nonpolar neutrals. Mutagenicity was demonstrated for the crude tar effluent, polynuclear aromatics, polar neutrals, and organic bases. The nonpolar neutrals and organic acids tested nonmutagenic for both tester strains employed in the bioassay. Some crude tars and chemical fractions exhibited substantial dose-responsive mutagenicity, but for certain coals the sum of the mutagenicity of the individual chemical fractions exceeded the mutagenicity of the crude tar, per unit mass. It was noted that certain fractions, especially organic bases, present as minor components per unit mass of crude tar, were highly mutagenic. The activity of such minor components would be masked when testing the mutagenicity of the crude tar.
Ac-11

MUTAGENICITY OF FLY ASH FROM A FLUIDIZED-BED COMBUSTOR DURING START-UP AND STEADY OPERATING CONDITIONS. H. E. Kubitschek and D. M. Williams*, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439.

Fly ash was obtained from an experimental, 6"-diam., atmospheric pressure fluidized-bed combustor operated by the Chemical Engineering Division at Argonne National Laboratory. This FBC burned high-sulfur (5.5%) bituminous coal (Sewickly) and used a calcitic limestone (Greer). Particle-culate effluent samples (fly ash) from the off-gas stream were collected in cyclones and on a final porous metal filter. Mutagenic activities of dimethyl sulfoxide extracts of these samples were determined with the Ames/Salmonella assay using strain TA98 without microsomal enzyme activation. Mutagenic activities were observed to depend inversely upon sample collection temperature with the largest values observed for samples from the filter, which was operated at a lower temperature than the cyclones. In addition, sample mutagenicity also depended upon operating conditions. In one run, samples collected during start-up and shut-down periods were approximately 60 times as mutagenic as samples collected during the period of steady operation. The very large activities during start-up account for most of the difference in activity of earlier samples from this bench scale model FBC and the samples obtained by others from larger units under steady operation. These findings also indicate the general requirement for comparing biological hazards from different coal technologies under steady operating conditions as well as for assessing contributions during start-up and shut-down of plant operation. (Work supported by the U. S. Department of Energy under contract #W-31-109-ENG-38.)

Ac-12

BIOLOGICAL AND CHEMICAL CHARACTERIZATION OF BAGHOUSE FLY ASH FROM A FLUIDIZED BED COMBUSTOR BURNING OIL SHALE. Antone L. Brooks, Ray Hanson and Ambrosio Sanchez, Lovelace Inhalation Toxicology Research Institute, P. O. Box 5890, Albuquerque, NM 87115.

The mutagenic and chemical properties of extracts from baghouse fly ash produced by burning oil shale in a fluidized bed combustor (FBC) have been evaluated. Three Ames tester strains (TA-98, TA-1538 and TA-100) were used to characterize the mutagenic activity. Tentative identification of the organics ultrasonically extracted from the ash with CH₂Cl₂ was done using GC/MS. The mutagenic activity detected with the most sensitive strain (TA-98) following ultrasonic extraction with the non-polar solvent CH₂Cl₂ ranged from 0.9 to 2.4 revertants/mg of ash. The mutagenic activity of the oil shale ash derived by extraction with the polar solvents such as dimethylsulfoxide (DMSO) ranged from 12-28 revertants/mg of ash. Partitioning of mutagenic activity out of the DMSO into a pentane and water phase resulted in mutagenic activity in only the non-polar pentane. No mutagenicity was detected in horse serum or dog pulmonary lavage fluid when up to 250 mg ash/ml was incubated for 1 week and the extract tested. Ultrasonic extraction of the same concentration with horse serum resulted in a positive mutagenic response. Several unique oxygenated and aliphatic hydrocarbons were present in CH₂Cl₂ extracts of the oil shale ash that were not found in analysis of CH₂Cl₂ extracts of ten different ash samples from FBC coal combustion. These compounds may, in part, be responsible for the higher mutagenic activity extracted with the polar solvents. (Research performed under U. S. Department of Energy Contract Number EV-76-C-04-1013.)
Mutagenicity Studies on Mice Chronically Exposed to Halothane E. Zeiger, D. Frezza*, J. Guthrie*, J. Allen and H. Mukhtar*. Laboratory of Molecular Genetics and Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) is a widely used general anaesthetic. It is not mutagenic in the standard Salmonella/microsome test, but urine of anaesthesiologists has been reported to be mutagenic for Salmonella and a presumptive metabolite has also been shown to be mutagenic for Salmonella. Male and female mice were exposed to Halothane (0.25% v/v, 6 hours/day, 5 days/week for 8 weeks) in inhalation chambers. The urines of treated mice, collected weekly throughout the entire exposure period, showed no mutagenic activity to Salmonella TA100 or TA1535. The intrasanguinous host mediated assay, using Saccharomyces cerevisiae D4 was run on the eighth week of halothane exposure; equivocal results were obtained. No enhancement of SCE's was seen in bone marrow of exposed mice. Liver homogenates (S-9) made after the ninth week of exposure showed significantly decreased levels of cytochrome P450 and arylhydrocarbon hydroxylase and a significantly increased level of epoxide hydrazine. Halothane was not mutagenic to Salmonella using halothane-induced S-9, however, benzo(a)pyrene mutagenesis was reduced.


Ultraviolet light and four chemical carcinogens having different enzymatic requirements for activation were tested for their ability to produce sister chromatid exchanges (SCEs) in HTC cells, a hepatic tumor cell line. Cyclophosphamide, N-nitrosodiethylamine (DEN) and aflatoxin B1 (AFB1) produced significant levels of SCEs without the use of microsomal enzyme preparations (S-9). Ultraviolet light and mitomycin C, a potent inducer of SCEs in all cell systems, displayed a marked and sensitive induction of SCEs. Sensitivity of HTC cells to DEN and cyclophosphamide was comparable to the most sensitive of other mammalian test systems. Exceptional sensitivity was found with AFB1, which produced a doubling of background SCE levels at a concentration of 10^-8M. The induction of SCEs by AFB1 was also shown to be inhibited by estradiol. A 50% reduction in the SCE level produced by 1.6 x 10^-7M AFB1 was achieved with estradiol at 10^-5M. It is known that estradiol inhibits activating enzymes and protects liver cells from aflatoxin induced cytotoxicity. SCE analysis shows the results of this interaction at the chromosomal level. The HTC-SCE system offers a simplified sensitive method of detecting carcinogens requiring activation. Moreover this system has the capability of demonstrating and investigating interactions between hormones and carcinogens.

Sister Chromatid Exchange Response of Murine Alveolar Macrophages, Bone Marrow, and Regenerating Liver Cells Following Inhalation of Urethane, M. Cheng*, M.K. Conner, Y. Alarie*. Department of Biostatistics and IEHS, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA 15261.

Murine lung and regenerating liver cells have been demonstrated to be highly susceptible to urethane induced neoplasias. We have utilized a recently described simultaneous multicellular in vivo assay (Conner et al, Chromosoma 74: 51-55, 1979) to evaluate the relative SCE response of alveolar macrophages, regenerating liver, and bone marrow cells of BDF1 mice.
following 4 hrs. exposure to aerosolized urethane solutions (0.5, 2.5, 5.0, and 10.0% w/v in H2O). At concentrations of 2.5% and above, urethane was found to be a potent inducer of SCE, producing concentration dependent increases in SCE frequencies in all cell types. At concentration levels of 5% and 10%, the mean frequency of SCE/cell was significantly lower (p< .01) in bone marrow (12.9, 20.6) than in alveolar macrophages (15.9, 26.3) or regenerating liver cells (16.2, 25.4) of hepatectomized mice. Comparisons of relative SCE frequencies were also made between similar cell types in hepatectomized and intact mice. At the 10% level, the mean frequency of SCE/cell in bone marrow (17.2; p< .02) and alveolar macrophage cells (23.1; p< .01) of intact mice were significantly lower than in the corresponding cells of hepatectomized mice. In nonhepatectomized mice bone marrow cells exhibited significantly lower (p< .01) mean frequencies of SCE/cell than alveolar macrophages at all urethane concentrations employed.

Ad-3
INFLUENCE OF METABOLIC POTENTIAL OVER BENZO(a)PYRENE INDUCED SCE - R.R. Schreck and S.A. Latt, Children's Hospital Medical Center, Boston, MA 02115

Detection of SCEs formed in vivo in cells from regenerating liver, the organ richest in activating enzymes, affords comparison of metabolic potential and drug-induced cytogenetic effects in different inbred mouse strains. To investigate this, mice were partially hepatectomized and injected, i.p. with 80 mg/kg 3-methylcholanthrene (3-MC) for enzyme induction; 48 hrs later, livers were collected for S-9 preparations to be used for enzyme assay, or benzo(a)pyrene (BP) was administered i.p., followed by BrdU exposure for SCE analysis on mitotic liver cells. Exposure of C57Bl/6 (inducible Ah+) mice to 3-MC caused a 6.8 fold increase in benzo(a)pyrene hydroxylase, while SCE frequencies in animals treated with 50 mg/kg BP were 9.6±0.9 SCE/cell (baseline 7.9±1.1) without induction and 12.0±1.8 SCE/cell (baseline 9.0±0.6) with induction by 3-MC. In contrast, exposure of DBA/2 (uninducible, Ah-) mice to 3-MC produced a 50% decrease in this enzyme activity, while the SCEs/cell observed in animals treated with BP was 10.9±1.1 (baseline 5.1±0.4) without induction and 16.3±1.2 (baseline 15.9±1.1) with 3-MC treatment. Thus no simple correlation was observed between fluorometrically measured levels of benzo(a)pyrene hydroxylase and SCE induction in response to benzo(a)pyrene. Similarly, preliminary data on bone marrow cells shows little correlation between enzyme induction (by 3-MC or phenobarbital) and cyclophosphamide-induced SCEs in these strains. The inability to predict drug-related SCE induction from single enzyme activity measurements may reflect the complexity of host mediated drug activation systems, and perhaps also interstrain differences in DNA repair.

Ad-4

The chemotherapeutic agent, BCNU, is used in the treatment of a variety of human and animal tumors, including the AKR lymphoma. In view of evidence suggesting an increased risk of development of secondary neoplasms as a complication of chemotherapy, it was the purpose of this study to evaluate more extensively the mutagenic/carcinogenic potential of BCNU. The frequency of sister chromatid exchanges (SCE) and kinetic analysis of BrdU labeled cells was used to assess DNA damage/repair and cellular toxicity produced by BCNU. Bone marrow cells of AKR/J and BDF1 mice were found to have similar baseline values of SCE/cell. However, over all concentrations of BCNU tested, AKR/J mice had a significantly lower frequency of induced SCE as compared to BDF1 mice. At 8.8 mg/kg
BCNU, the mean frequency of SCE/cell for AKR/J was 23.22±1.48, and 28.01±1.70 for BDF₁. BCNU was found to produce significant changes in the proportions of first, second and third division metaphases. An increase in the average proportion of first division metaphases (from 11% to 52%) was accompanied by a decrease in the average proportion of second division metaphases (from 79% to 49%) and a decrease in the average proportion of third division metaphases (from 10% to 0%) over the range of doses employed. There were no significant differences in the proportions of these metaphase types between AKR/J and BDF₁ mice. BCNU was effective in producing dose-related increases in SCE frequency in bone marrow cells of both AKR/J and BDF₁ mice. The lower response of AKR/J mice to BCNU suggests a strain difference in susceptibility to certain drugs, and/or a decreased ability to repair specific types of DNA damage.

Ad-5

DEMONSTRATION BY SISTER CHROMATID EXCHANGE OF GENETIC DIFFERENCES IN BENZO(α)PYRENEx METABOLISM IN CULTURED MOUSE EMBRYOS AT EARLY GESTATIONAL STAGES S.M. Galloway¹*, P.E. Perry¹,², J. Meneses¹*, D.M. Nebert³, and R.A. Pedersen¹*. ¹Lab. of Radiobiology, San Francisco, CA; ²MRC Cytogenetics Unit, Edinburgh, Scotland; ³Developmental Pharmacology Branch, NIH, Bethesda, MD; ⁴Present address: Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, MD 20795.

We used sister chromatid exchange (SCE) as an endpoint to detect metabolic activation of benzo(a)pyrene (BP) in mouse embryos (7.5 and 8.5 days of gestation) cultured without maternal tissue. The murine Ah locus regulates the inducible capacity to metabolize BP. We found that SCE induction by BP in vitro correlates with the Ah status of embryos. A high frequency of SCE is induced by BP in embryos from three Ah-responsive inbred strains, Balb/cDib, C3H/AnFcum, and C57Bl/6n (e.g. 37 SCE/cell at 1 μM BP cf 7.2 SCE/cell in controls.) There is much less BP-induced SCE in embryos of two Ah-non-responsive strains AKR/J and DBA/2J (e.g. 13 SCE/cell at 1 μM BP cf 6.6 SCE/cell in controls.) BP also induces SCE in the Ah-responsive recombinant inbred line B6NXAKN-12, but not in the Ah-nonresponsive recombinant inbred line BGNXAKN-3. These data indicate that the Ah regulatory gene products are functional from an early embryonic age, in keeping with a recent demonstration [Shum, et al, Teratology, in press (1979)] that BP-induced birth defects and in utero toxicity can be mediated by fetal, rather than maternal, drug metabolism. Further, SCE in cultured Ah-responsive mouse embryos is shown to be a sensitive assay for environmental mutagens.

Ad-6


An important part of toxicological screening of chemicals is the assessment of potential mutagenicity to cells of the developing embryo. In this regard, the early chick embryo represents a compact in vivo system for investigating the mutagenic actions of chemicals at defined periods of development. Of particular interest is the question of whether early embryos can metabolically convert indirect-acting compounds to mutagenic forms. If this is the case, increasing amounts of such mutagens would be formed as development advances. To investigate these points, the sister chromatid exchange (SCE) technique was used as a mutagenic indicator at two stages of incubation in the chick, day 3 and day 6. At these two stages, embryos were exposed to the same graded series of dosages of aflatoxin B₁, 2-acetylaminofluorene, and ethyl methanesulfonate (EMS)(pos-
itive control) for a period of 22 hours. All three mutagens were effective in increasing the frequency of SCE above the control rate of 1.8 SCEs/cell. While a dose-dependent increase in SCE was obtained for both pro-mutagens at each age, the mean SCE frequency was significantly higher in the 6-day embryos for each dosage given. In contrast, the direct-acting mutagen, EMS, gave a reduced level of SCEs at the older age. These results are reflective of the ability of early chick embryos to activate pro-mutagens to forms capable of inducing SCE. As the embryo develops and differentiation of cells proceeds, this conversion is apparently enhanced so that increased SCE occurs in cells of the 6-day embryo. These results suggest that the genetic hazards of indirect-acting environmental mutagens may be greater with advancing developmental age.

Ad-7
IN UTERO ANALYSIS OF SISTER CHROMATID EXCHANGE: DIFFERENTIAL SENSITIVITY OF FETAL TISSUES TO MUTAGENIC DAMAGE. David Kram, Gaither Bynum, Gerhard Senula*, Charlene Bickings* and Edward Schneider*, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224.

The majority of teratogens show specificity in their effects for particular organs and stages of gestation. This can be explained by the rapid growth of particular organ systems at specific times in development or by the presence of particular enzyme systems in the target tissues. We have recently described a short term assay for detecting potential teratogens (which act at the level of DNA damage) through the analysis of sister chromatid exchange (SCE) in fetal and maternal tissues (Nature 279, 231, 1979). The present study was designed to determine if different fetal cell types display differential sensitivity to chemical mutagenesis. Pregnant C57BL/6J mice are intravenously infused with bromodeoxyuridine at a rate of 50 mg/kg wt/hr for 24 hrs. Test compounds (cyclophosphamide, CP, 10 mg/kg; mitomycin C, MMC, 1 mg/kg; daunomycin, DNM, 5 mg/kg) are injected intravenously one hr after the onset of infusion. Drug concentrations were chosen to induce approximately 20 SCE/cell in maternal bone marrow. CP induced the highest level of SCE in fetal tissues followed by MMC, while DNM induced only 7 to 8 SCE/fetal cell. Comparisons of the different fetal tissues revealed that the direct acting mutagens, MMC and DNM induced equal levels of SCE in all cell types. CP, which is metabolically activated induced significantly higher levels of SCE in fetal liver (36 SCE/cell) than in lung (21 SCE/cell) or gut (25 SCE/cell). Since in utero SCE analysis can be performed between day 11 and 19 of gestation, this system can be used to determine which fetal organ systems demonstrate sensitivity to particular drugs at specific times in development.

Ad-3
INDUCTION OF MUTATIONS AND SCEs FOLLOWING S9 ACTIVATION AND DEACTIVATION OF PROMUTAGENS IN CULTURED MAMMALIAN CELLS. R. T. Okinaka, G. F. Strniste, D. J. Chen and B. J. Barnhart, Genetics Group, University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545.

The induction of both mutations and sister chromatid exchanges (SCEs) was measured in cultured Chinese hamster cells (line CHO) using added rat liver homogenate (S9) to activate promutagens. In the presence of fixed levels of S9, the activation of dimethylnitrosamine (DMN) and benzo(a) pyrene (BP) caused a linear dose response in the formation of both SCEs and mutations in the hgpri locus. Cytotoxicity measurements, when BP concentrations were held constant and the S9 levels were varied, revealed an initial cytotoxic effect at lower levels of S9 followed by a detoxifying effect at high S9 concentrations. This was also reflected by SCE
and mutation measurements which were highest at the maximum killing levels. The degree of BP metabolism (as measured by extraction with organic solvents) indicates that as S9 concentrations are elevated the rate at which BP is converted to water soluble products increases. One interpretation of these results suggests that rapid BP metabolism at high S9 concentrations prevents biologically reactive intermediates from accumulating in sufficient quantities for SCE or mutational events to occur. These studies indicate that under conditions suitable for growth of CHO, metabolic activation and deactivation of a PAH leads to parallel induction and deinduction of SCEs and mutations. [This work was performed under the auspices of the United States Department of Energy.]

Ad-9


In this study steroids were evaluated for their ability to modulate the capacity of known mutagenic agents to induce sister chromatid exchange (SCE). Transformed Chinese hamster lung (CHL) fibroblasts were cultured in eagles minimal essential media supplemented with fetal calf serum (Flow, 10%), glutamine, tetracycline (Gibco, 50μg/ml) and 2-bromo-5-deoxyuridine (BrdU, Sigma, 10μg/ml). CHL cells were cultured for 2 hours in the presence of the steroids (Sigma, 10^-5M), dehydroepiandrosterone (DHEA), estradiol, progesterone, and testosterone prior to exposure to the mutagens, mitomycin C (GMC, Sigma, 2.5ng/ml) and ultraviolet light (UV, 120ergs/mm²). After two cell replications, colcemid (Sigma, 200ng/ml) was added for 2 hours, cells dislodged with pronase (Gibco, 0.1%), fixed (methanol-acetic acid, 3:1) on slides, and stained (DAPI, 10μg/ml). Data demonstrate that SCE induction by mutagens may be selectively modulated by extracellular factors such as steroids. The modulation of UV light SCE induction suggests that mechanisms other than mutagen metabolism or transport must be considered. It is unclear whether the steroid effect is a reflection of enhanced DNA vulnerability or an enhanced DNA response (SCE) to a constant mutagen load.

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Estrad.</th>
<th>DHEA</th>
<th>Proges.</th>
<th>Testos.</th>
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<td>Control</td>
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<td>13.2±0.9</td>
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<td>50.8±5.9</td>
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</tbody>
</table>

* SCE/metaphase cell; 15 metaphase cells per data point

Ad-10

A COMPARISON OF METABOLIC SYSTEMS TO ACTIVATE CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN VITRO FOR SISTER CHROMATID EXCHANGE. P.S. Sabharwal, J.P. Wojciechowski, Parvinder Kaur. Thomas Hunt Morgan School of-Biological Sciences, University of Kentucky, Lexington, KY 40506.

Rat liver homogenate (S-9 Mix) and irradiated Syrian hamster fetal cells (feeder layer) were used to compare their ability to metabolize three known carcinoogenic polycyclic aromatic hydrocarbons. The metabolic activity was determined by comparing the frequency of sister chromatid exchanges (SCE) induced in V79 cells. The S-9 (1/20th strength) Mix was cytotoxic to the target cells even at a low exposure time of 2.5 hours. In controls, the mitotic index decreased from 0.036 to 0.019 when S-9 Mix was incorporated, even without the test chemical. The target cells in the presence of the feeder layer had a mitotic index of 0.0395, which is very similar to controls without an activating system. Since the irradiated feeder layer was not cytotoxic it was incorporated for 28 hours, the duration of the experiment. This allowed a longer exposure period of test chemical and
activating system to the V79 target cells. Both activating systems elicited a dose response in terms of induction of SCE by the polycyclic aromatic hydrocarbons. For DMBA a plateau effect was observed at concentrations of 0.01 and 0.05 μg/ml in the presence of the S-9 Mix. At similar concentrations of the carcinogens, there was a significant increase in the frequency of SCE when feeder layer was used as compared to the S-9 Mix. For example, with BaP at 0.01 μg/ml SCE increased by 17% when feeder layer was used as compared to the S-9 Mix. Similarly at 1 μg/ml of 3-MC and 0.05 μg/ml of DMBA an increase of 46% and 63% of SCE was observed. From these studies we conclude that the feeder layer is a preferred activating system since it was non-toxic to the target cells and the known carcinogens induced higher frequencies of SCE as compared to the S-9 Mix.

Ad-11


In the search for relevant systems for mutagenicity and carcinogenicity testing, there has been little development of systems using human tissues and cells. Investigators have preferred to work with well-established Chinese hamster ovary (CHO) cell lines with their short cell cycle, excellent growth characteristics and long lifespan in culture. However it may be important to use the more difficult human cell strains if it is found that they do not respond to chemical treatment in the same manner as CHO cells. To investigate this possibility we have treated human diploid cells (IMR-90, WI-38, S-3299) and CHO cells with the direct-acting mutagen, mitomycin C (MMC), and two indirect-acting mutagens, dimethylnitrosamine (DMN) and benzo(a)pyrene (BP) using a standard metabolic activation procedure. Our biological end-point was the induction of sister chromatid exchanges (SCE). Both human and CHO cells showed a significant dose-response to MMC, DMN and BP but there were significant differences in the magnitude of the response. While human and CHO cells showed a similar dose-response to BP resulting in a doubling of SCE at doses which caused a 50% reduction in the proportion of cells in second mitosis, the responses to the alkylating agents, MMC and DMN, were different. The CHO cells showed a two-fold increase in the frequency of SCE compared to the human cells. There were only slight differences among the human cell strains in response to DMN and BP. The observed differences between human and CHO cells may reflect differences in the fate of metabolic intermediates of MMC and DMN.

Ad-12

FRANK CHROMOSOMAL BREAKAGE AND METHYLAZOXYMETHANOL ACETATE. L.A. Evans,$^1$, C.J. Duncan$^2$, E.C. Jenkins$^2$, $^1$Hedgar Evers College of CUNY, Brooklyn, N.Y. 11225 and New York State Institute for Basic Research in Mental Retardation, Staten Island, N.Y. 10314.

Several studies have established the mutagenicity and carcinogenicity of methylazoxymethanol acetate (MAM AC). In comparison to "frank" chromosomal breakage studies sister chromatid exchange (SCE) is considered a more sensitive indicator of chromosomal damage. The present study asks the question as to whether or not MAM AC exerts an effect on frank chromosomal breakage in short-term human leucocyte cultures derived from the same individuals and exposed to the same concentrations of MAM AC which, in our earlier work (Mut. Res., 56:51), caused increased frequencies of SCE. A total of 1408 metaphases was evaluated in a single blind analysis for the incidence of chromosomal aberrations. All cells contained 46
chromosomes. Only single chromatid breaks were observed. Statistical analyses of the data have indicated that there were no significant differences in the incidence of chromatid breaks when cultures treated with MAM AC were compared to control cultures (p>.2). Therefore MAM AC has no effect on the incidence of frank chromosomal breakage and SCE is a more sensitive indicator of chromosomal damage than frank chromosomal breakage is in this system. Our results agree with other reports stating that SCE and frank chromosomal breakage may result from different mechanisms. It has been hypothesized that the production of frank chromosomal breakage is associated with cell death. In this study, based on dose-effect curves, we used concentrations of MAM AC which did not suppress blastogenesis. This work was supported by NSF Grant No. 00521 and N.Y.S. O.M.R.D.D.

Ad-13


The conditions for growing cells and for staining in the SCE assay were optimized and standardized so that reproducible and permanently stained sister chromatid differentiation in V-79 (Chinese hamster) cells were obtained. We observed adequate sister chromatid differentiation, with minimum growth inhibition, at 2.5 μg/ml BrdU. The interaction of Hoescht dye, light exposure, and Giemsa staining was optimized for 2.5 μg/ml BrdU. The reproducibility of the assay was established in 4 separate experiments in which cells (quadruplicate flasks) were exposed for 2 hrs. to 0, 3 x 10^-8, 10^-7 and 3 x 10^-7M MMC. At these doses of MMC, cell survival (based on cloning efficiency) was at least 90%. The mean exchange rate of 4 slides per experiment range from 8.77 to 10.66/cell for controls and 15.46 to 18.7/cell for 3 x 10^-8M MMC. The null hypothesis, that 3 x 10^-8M MMC group has the same or smaller mean exchange rate than the control group is rejected in all 4 experiments (p=0.000 to 0.005). The anthraquinones, adriamycin and 7-OMEN are highly effective against various tumors in vivo. Adriamycin and 7-OMEN induced SCE in a dose-related fashion. Although the maximum increase in SCE with adriamycin and 7-OMEN was respectively 4-fold and 2-fold that of the untreated controls, the increased SCE were obtained at drug exposures (0.3 μg/ml, 2 hrs.) that killed 80% of the cells. Chromosome aberrations were observed with both drugs.

Ae-1

ACTIVATION OF PROMUTAGENS TO BACTERIAL MUTAGENS BY LIVER POST-MITOCHONDRIAL (S-9) FRACTIONS AND TWO HEPATOCYTE PREPARATIONS. D. M. Zimmer, J. H. Mazurek, G. L. Petzold, and B. K. Bhuyan, The Upjohn Company, Kalamazoo, MI

The hepatocyte primary culture/DNA repair system detects structurally different carcinogens that require metabolic activation. This report compares the activating ability of hepatocytes prepared by liver-perfusion or -mince techniques to that of S-9 preparations. Activation was measured by the ability of the promutagens [aflatoxin B1 (AFB1), benzo(a)pyrene (B(a)P), 7,12-dimethylbenzanthracene (DMBA), 2-acetylamino-fluorene (AAF), 4-aminobiphenyl (ABP), 3-methylcholanthrene (MCA) benzidine, and acridine orange (AO)] to cause mutations in S. typhimurium. For this purpose, the hepatocytes (in HBSS) or S-9 preparations were incubated with bacteria (TA98) in suspension for 1 hr at 37°C following
which they were plated on minimal agar medium. AAF, ABP and MCA were mutagenic when incubated with either S-9 or perfused hepatocytes. AAF gave variable results in the hepatocyte system (600 to 3400 revertants/μmole) as compared to about 4500 revertants/μmole in the S-9 system. ABP and MCA were strongly mutagenic with S-9 (900 and 2500 revertants/μmole, respectively) but were much less mutagenic with perfused hepatocytes (160 and 470 revertants/μmole, respectively). AO and benzidine were mutagenic with S-9 but nonmutagenic with perfused hepatocytes. AFB; and DMBA were also mutagenic with perfused hepatocytes whereas B(a)P was nonmutagenic. Activation by perfused or minced liver hepatocytes appeared to be similar. The implications of our results for the activation of promutagens in other mammalian cell assay systems (V79 mutation or sister chromatid exchange) will be discussed.


We have previously reported that, although dimethylnitrosamine (DMN) is not mutagenic in the standard Salmonella plate incorporation (Ames) assay using a metabolic activation system (S-9) derived from rat liver, the mutagenicity of DMN can be demonstrated if the S-9 is derived from mouse or Syrian golden hamster liver (Environ. Mutagen., 1, 95, 1979).

DMN is thought to be activated to its mutagenic form by DMN demethylase, a microsomal enzyme. We found that 10 micromoles or less of DMN per plate is not mutagenic in the presence of mouse S-9, but is highly mutagenic with hamster S-9. However, the DMN demethylase activity of the mouse S-9 is similar to that of the hamster S-9. Rat liver S-9 has less enzyme activity than either mouse or hamster S-9. Furthermore, both mouse and rat microsomes can inhibit the mutagenic activity of DMN in the presence of hamster S-9, although they do not inhibit the hamster enzyme activity.

Under the conditions of our assays, hamster microsomes are not sufficient to mediate DMN mutagenicity. The cytosolic fraction (S-105) from hamster, mouse or rat S-9 must be added to hamster microsomes to observe good mutagenic activity. S-105 itself has no detectable DMN demethylase activity and has little or no effect on the activity of hamster microsomal DMN demethylase. It is concluded that constituents of S-9 other than DMN demethylase can affect the mutagenicity of DMN in the plate assay.


We have shown that mutation induction by polycyclic aromatic hydrocarbons (PAHs) in L5178Y mouse lymphoma cells decreases as the concentration of an exogenously added activation system (S-9) is increased. Studies of the metabolites formed from benzo(a)pyrene (BaP) indicate that this decrease in mutation frequency may result from rapid oxidation of the hydrocarbon to non-mutagenic metabolites. We now report the effect of S-9 concentration on BaP-DNA adduct formation. (3H)-BaP (3 μg/ml) was incubated with cofactors, 2.5 or 5.0 ml S-9, and either calf thymus DNA or intact L5178Y cells in a total volume of 10 ml. The DNA was then isolated, enzymatically degraded to deoxyribonucleotides, and analyzed by high pressure liquid chromatography. At the low S-9 concentration 33 pmole of
(3H)-BaP were bound per mg calf thymus DNA, whereas only 15 pmole/mg were bound at the high S-9 concentration. The major BaP-DNA adduct in both calf thymus DNA and DNA isolated from intact L5178Y cells was the anti-BaP 7,8 diol-9,10 epoxide-deoxyguanosine adduct. At the low S-9 concentration, 12 pmole of this adduct were bound per mg calf thymus DNA compared to 2 pmole/mg at the high S-9 concentration. These results suggest that high concentrations of S-9 rapidly oxidize the hydrocarbon to secondary metabolites which do not react with those target sites in the cell which are necessary for mutation. This study also demonstrates the value of this system for studying the mechanism of mutation induction by PAHs since the BaP-DNA adducts produced under these conditions are identical to those found in cells in culture and tissues in vivo. (Supported by NIH Grants CA 09171, CA 19448, CA 08936, and CA 21778).

DURATION OF S-9 ACTIVITY. M.S. Kelley, J.M. Baden, Veterans Administration Hospital, Palo Alto, CA 94304.

Many currently used fluorinated anesthetics have been tested for mutagenicity using the Ames-Salmonella/mammalian microsome system. The anesthetics are metabolized by mixed function oxidases which are added to the test system usually in the form of a rat liver homogenate. Previous mutation assays included such a metabolic system (S-9) with Salmonella typhimurium either in liquid suspension for 2 hours or on plates in desiccators for 8 hours. It was assumed that the S-9 metabolized the anesthetic throughout the test period. This study examines that assumption by determining the extent of S-9 activity in a system which simulates both the liquid suspension and desiccator assays. S-9, derived from Aroclor 1254 pretreated rats, was added to either a liquid buffer solution, soft agar, or a bacterial suspension in nutrient broth and incubated at 37° for up to 24 hours with an excess of the volatile fluorinated anesthetic, methoxyflurane (MDF). Inorganic fluoride (F⁻), a metabolic end product of MDF, was measured by an Orion ion specific electrode. Control samples were prepared in a similar way but without MDF and standards containing known F⁻ concentrations were included in each experiment. The amount of F⁻ released by the S-9 reach a maximum by 2-3 hours in buffer and by 6 hours when incubated in soft agar. Addition of bacteria did not affect the accumulation of F⁻. These results show a differential rate of activity of S-9 in liquid versus a semisolid (soft agar) medium agreeing with an earlier report (1). Moreover, the period of S-9 activity was limited since the amount of F⁻ produced with time reached a maximum and then plateaued.


Ac-5

The NCTR "Microlesion Assay" utilizes the assay of 20-30 enzyme activities as genetic "markers" for detecting mutations in mice (Feuers, et al, Mutat. Res. 53;99, 1977). The variance of any enzyme analysis is directly associated with the ability to detect induced mutations. The components of variance in studies conducted over extended periods where multiple enzymes are analyzed in large populations are technical, biological and genetic. Circadian rhythm studies have been completed in which sacrifices, sample preparations, and analyses were all performed with
concurrent pooled controls as standards. All enzymes within the battery were found to be subject to rhythmic, quantitative change (circadian rhythms). As typical examples, the temporal organization of isocitrate dehydrogenase (ICD) and glutathione reductase (GR) are cosine curve fits and during time intervals of accelerated change, activities decrease 11.08 and 8.54% per hour, respectively. By employing these sample preparations and analysis techniques for animals killed at an activity plateau, coefficients of variation were decreased from 0.23 for both enzymes to 0.09 and 0.05 (a decrease in variance of 61 and 78%), respectively. Implementation of these analysis parameters will reduce all sources of enzyme activity variance, and remaining variance will more accurately reflect biological and genetic differences.

Ca-1
THE GENOTOXIC EFFECT OF ADRIAMYCIN IN SOMATIC AND GERMINAL CELLS OF MICE.
William Au, and T. C. Hsu, intr. by F. E. Arrighi, Department of Cell Biology, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Adriamycin (ADR) is one of the most effective and commonly used anticancer drugs. However, it has been classified as a carcinogen and it is extremely cytotoxic. The genotoxic effect of ADR on somatic and germinal cells in vivo were analyzed using cytogenetic assays. Young adult Swiss mice were treated with 1 dose of ADR (IV or IP) ranging from 0.5 – 24 mg/Kg body weight. Animals were sacrificed on 1, 3, 5, 9, 11 and 15 days PI. It was observed that animals treated with high doses (3-24 mg/Kg) invariably showed testicular atrophy at approximately 15 days. Chromosome breakage frequency in diakinesis-metaphase I and metaphase II showed a dose-dependent increase. The damage peaked at 3 days which suggested that the meiotic stage at late pachytene was very sensitive to ADR-induced chromosome breakage. In comparison, the damage in the bone marrow cells was highest at 24 hours PI and there was no significant mitotic suppression in this hematopoietic system. Among the mice treated with lower doses of ADR, recovery of gametogenesis occurred at approximately 60 days PI. Mice were sacrificed at 10-day intervals up to 100 days. Chromosome damage was observed at diakinesis-metaphase I. Ring IV and chain IV were found which were characteristic for damage induced in spermatogonial cells and observed in spermatocyte I. Our data shows that ADR can induce genetic damage that may be passed on from one generation to another. It implies that patients treated with ADR before or during their reproductive age may be subjected to additional genetic burden.

Ca-2

Leopard frogs, Rana pipiens, have been shown to have potential value in mutagenesis studies of dominant lethality. Unfortunately, R. pipiens with mature gametes are unavailable during summer months. It was the purpose of this study, therefore, to ascertain whether or not the South African clawed toad, Xenopus laevis, which has continuously available mature gametes, would be similarly useful for mutagenesis research. X. laevis, like R. pipiens, produces hundreds of ova per ovulation. In vitro observation of fertilization, cleavage, and subsequent embryonic development is possible. Chromosomes are easily recovered from embryos with short term culture. Male X. laevis were injected IP with TEM that varied from 13 to 1300 ng/kg. Sperm from treated males was used to
inseminate ova of normal females. Fertilization rates from treated males were not significantly different from control rates. Lethality during the first 7 days of development was dose related. Also, developmental abnormalities, impaired swimming capabilities ($R^2=0.92$, $R^2=0.94$ respectively) and cytogenetic aberrations (rings, dicentrics, complex exchange figures, etc.) were linearly dose related. We conclude that X. laevis merits additional study with other classes of mutagenic chemicals because of its demonstrated sensitivity to TEM-induced mutation. The test system permits correlation of dominant lethality with chromosomal aberrations. Further, it is possible to compare induced somatic chromosomal mutations with induced genetic chromosomal mutations for a given chemical agent. Supported by NIH Grant 5R01-ES 01605-02.

Ca-3
STUDIES ON INDUCTION OF DOMINANT-LETHAL MUTATION AND HERITABLE TRANSLOCATION WITH ETHYLENE OXIDE IN MALE MICE. W. M. Generoso, C. W. Sheu, and R. M. Cryder, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN and Bureau of Foods and National Center for Toxicological Research, Food and Drug Administration, Washington, DC.

Ethylene oxide was studied for induction of dominant-lethal mutations and heritable translocations in male mice. The chemical was prepared in water and injected intraperitoneally. The dominant-lethal study was conducted using a single injection of 150 mg/kg (maximum tolerated dose), while in the heritable translocation study males were injected daily on weekdays for five weeks with 60 or 30 mg/kg per day. Results clearly show that ethylene oxide is effective in inducing dominant-lethal mutations in treated males and that the four stocks of untreated females used in the study did not differ in the ability of their eggs to repair ethylene oxide-induced lesions. The affected stages are early and midspERMatozoa and late spermatids. Clear-cut increases in the frequency of heritable translocations were also induced by the ethylene oxide treatments. At the 60 and 30 mg/kg/day doses, 9.36 percent (38 in 406 progeny tested) and 1.32 percent (6 in 456 progeny tested) heritable translocations were observed, respectively. Both frequencies are significantly higher than the contemporary control frequency of 0 translocation in 822 progeny tested. The frequencies observed at the two doses do not deviate significantly from those expected on the basis of dose-square kinetics. [Research jointly sponsored by the Food and Drug Administration and the Department of Energy under contract with the Union Carbide Corporation.]

Ca-4

Studies are in progress at the NCTR to identify, evaluate and, where possible, improve those components of the HTA which affect its precision as applied to the investigation of mutagenic compounds subject to regulatory risk assessment. Variance in the litter size of "tester" females used in the "Sequential Test Method" (Generoso, et al, 1979) has been identified as a critical statistical element affecting the false negative or b-error associated with classification of an F1 male. The larger this classification error, the larger the overall false negative test error ($\beta$) and, consequently, the larger the F1 test population required to maintain a given "Power of Test". Assuming that the average litter size for any population of translocation heterozygotes (TM's) is reduced from normal by a factor, $\alpha=0.5$, and the standard deviation reduced by a factor, $\pi=0.5$, the b-error $(b_i)$ associated with their detection by mating with "tester" females of a stock, i, having mean litter size, $\mu_i$, and standard deviation,
$\sigma_1$ is defined as the probability that the litter size, $i$, of a particular random mating will be larger than the defined decision value, $k_1$; i.e.,

$$b_i = P(\frac{\mu_1}{\sigma_1} > k_1) = \frac{\mu_1 - \mu_0}{\sigma_1};$$

given a true TH male. By equalizing a-errors in a simple one-stage screen at $< 0.05$, b-errors for four stocks of "tester" females with $\mu_1/\sigma_1/k_1$ of 14.0/3.3/8, 9.9/2.1/6, 10.9/1.7/8 and 12.4/2.3/8, were estimated to be 0.272, 0.159, 0.001 and 0.059 respectively. These results underscore the importance of variability of litter size in stock selection in that significantly smaller b-errors are associated with smaller variance. This differential effect in classification error for a more complex screen and its impact upon the "Power of Test" will be discussed.

Ca-5

MUTAGENICITY AND CYTOTOXICITY OF N-CHLOROPIPERIDINE. Bempong, M.A. and F. Scully*, Norfolk State University, Norfolk, VA 23504

Chinese hamster ovary (CHO) cells were exposed to varying concentrations of N-chloropiperidine (NCP) for 3 or more hr and harvested at different recovery periods for cytological analysis. Dose- and time-response curves for the cytotatic effect of NCP demonstrated a drastic reduction in percent cells with mitotic activity as NCP concentration increased. Analysis of control and treated cells for mitotic aberrations revealed that NCP-treated cells were characterized by abnormal nuclear division and distribution. The principal anomalies in nuclear division consisted of multipolar anaphase and ana-/telophase bridge-fragment configurations and laggards. Errors in nuclear distribution were mostly bi- and poly-macro-nucleation and mono-, bi and poly-micronucleation. Results of the cytogenetic studies showed that NCP-treated cells had a significant increase in chromosomal structural anomalies over those occurring in the control population. Exchange configurations, fragments and chromatid breaks were evident. CHO cell population exposed to NCP were also characterized by centromeric exaggeration. It may be concluded from the data that (1) NCP is a potent cytotatic, cytotoxic and clastogenic compound; (2) the observation of induced exchange configurations is a significant cytogenetic finding since such errors lead to stable chromosomal aberrations and are thus capable of producing genetic variations if transmitted from one generation to another.

Ca-6


Formaldehyde is an important industrial chemical produced and released into the environment in substantial quantities. Preliminary assessments of carcinogenicity studies in rodents suggest some neoplastic activity. Paraformaldehyde was evaluated in the Ames assay, the L5178Y mouse lymphoma assay (TK locus forward mutation), and for SCE's and chromosome aberrations in CHO cells. All tests were conducted with and without a hepatic activation system. The mouse lymphoma and the SCE assays showed significant dose-related increases in the induction of these endpoints. Activity was obtained both with and without the activation systems. However, evidence for metabolic modulation of the genetic activity was observed. No positive effects were obtained with the Ames test and CHO transformation assay is currently in progress and the results will be reported.
Ca-7


Previous analysis of over 7,000 metaphases from 90 rabbits demonstrated that the radiomimetic clastogen, streptonigrin (SN), induced dose-related chromosome lesions in lymph node (LN), bone marrow (BM), and oocytes. The oocyte aberrations were observed as inherited structural chromosome lesions in 13 out of 278 6-day blastocysts from females treated with various doses of SN prior to mating. This is in contrast to a control frequency of 0 abnormal blastocysts out of 91. Based on the slope coefficients derived from linear regression analysis, the lesion frequency in LN and BM cells from SN treated rabbits over-estimated by a factor of 2 and 6, respectively, the number of blastocysts with structural lesions. To determine if this reduction in lesion frequency between somatic and germ cells was related to the mechanism of SN's clastogenic action, a similar comparison was made following treatment with the alkylator, cyclophosphamide (CP). A linear dose response relationship was obtained for lesion induction in over 1,600 LN and BM metaphases from rabbits injected i.p. with doses of 12.5-100.0 mg/kg CP; as with SN, the aberration rate in LN was higher than in BM. For blastocyst analysis, females were given 75 mg/kg CP and then mated. Currently, 80 blastocysts have been collected and a minimum of 3 karyotypes have been prepared from 43 of these. One blastocyst with a structural aberration has been identified. Based on these data, the reduction in lesion frequency from somatic cells to 6-day blastocysts cannot be declared to be significantly different for these two chemicals. (Supported by U. S. Department of Energy and NICHHD Grant #HD-08828.)

Ca-3


While there is a general correlation between the effects of mutagens on chromosomal integrity and movement, on the one hand, and SCE formation and specific locus mutation, on the other, the correlation is far from absolute, and, particularly for weak or intermediate strength mutagens, may be nonexistent. For our studies with Methadone, Naltrexone, and L-alpha-acetyl-methadol (LAAM) on cultured human lymphocytes, we selected two continuous cell lines (Wi-L2 and GM130), used a variety of positive control compounds (including EMS, ICR 191, and caffeine), and determined the LD50 for each drug in these cell lines. Doses above and below the LD50 were used in the experiments. We demonstrated for each of these compounds, used in treatment of narcotic addiction, that: a) none of them causes an increase in the frequency of forward mutation at the hpt locus; b) they all produce minimal effects on SCE frequencies; c) they all exert intermediate level effects on chromosome segregation; and d) Naltrexone and LAAM induce significant breakage. These findings suggest classification of the three compounds as mutagens of intermediate potency in vitro in these human cells.

A series of chemical carcinogen/non-carcinogen pairs were tested for the induction of large colony trifluorothymidine-resistant mutants in a modified mouse lymphoma cell TK<sup>/</sup>− + TK<sup>/</sup>/− assay. These modifications consisted of a reduction of serum content in both growth and cloning media to 5%, omission of sodium pyruvate, and a decrease by one-half of the liver S9 cofactor levels previously described (Clive et al., Mutation Res. 59:61, 1979). S9 was from either non-induced Sprague-Dawley rat, ICR mouse, or Syrian hamster. Paired chemicals included representative epoxides, alkyl halides, aromatic amines, polycyclic hydrocarbons, nitroaromatics,azo compounds, and some Ames negative carcinogens. The use of 5% serum for soft-agar cloning increased the necessary interval between plating and colony counting from 7 to 10 days. Positive mutagenicity was detected for such procarcinogens as 3-methylcholanthene (p<0.01), 2-aminofluorene (p<0.01), and azobenzene (p<0.01). Non-carcinogens such as p-aminophenol, 1-naphthol, and methyl orange were negative when tested with rat S9. Benzene(ene)pyrene was clearly positive (p<0.01) with both rat and mouse S9 and weak activity was detected when urethane was tested with rat S9. Collectively, these results indicate that serum and S9 levels can be lowered considerably without affecting the validity of this mammalian cell assay.


Currently one fourth of the population will develop cancer. Antineoplastic drugs are frequently used for the treatment of the cancer. Anticancer drugs are also being used for treating certain non-neoplastic diseases such as psoriasis and rheumatoid arthritis. Paradoxically, many of the clinically useful anticancer drugs are carcinogenic in rodents. This obviously is quite disturbing to the clinical oncologist, since there is a distinct possibility of cancer patients receiving cancer chemotherapy developing a second neoplasm later in the life especially if the patient is young. Many of the antitumor agents such as cyclophosphamide, bleomycin, adriamycin, cis-platinum, methotrexate, and others have been shown to be mutagenic for mammalian cells in culture. Most of the drugs except few such as bleomycin and methotrexate were mutagenic in Ames Salmonella reverse mutation assay. The lack of correlation between the mutagenicity in Ames test and the mutagenicity in mammalian cells was attributed to the inability of prokaryotic cells (Ames Salmonella strains) to detect certain kinds of genetic damages such as translocations and chromosomal non-disjunction. In this presentation, we report that by using Rosenkranz's E.coli pol A<sup>+</sup>/pol A<sup>−</sup> DNA repair test and Saccharomyces cerevisiae D4, we can detect the genetic activity of some drugs which are non-mutagenic in Ames test. Research supported in part by American Cancer Society Grant # IN-132.
Ca-11
MUTAGENESIS OF COAL FLY ASH LINKED TO A TRACHEAL GRAFT ASSAY FOR CARCINOGENESIS. C. E. Chrisp and G. L. Fisher, Laboratory for Energy-Related Health Research, University of California, Davis, CA 95616

The tracheal transplant technique involving beeswax pellets containing carcinogens (Griesemer et al. Tracheal Grafts. In E. Karbe & J. F. Parks (eds), Experimental Lung Cancer: Carcinogenesis and Bioassays, 1974, pp. 539-547.) has proven useful in relating carcinogen release rate to carcinogenesis in rat tracheal transplants. When tracheas containing these pellets are implanted subcutaneously in syngeneic rats, carcinomas were found in the epithelium of the transplanted trachea. We have devised a modification of this technique which we believe is applicable for the study of potential carcinogens on the surface of coal fly ash. To eliminate the possibility of solid state carcinogenesis from particles which come into direct contact with tracheal epithelium, we have made packets from nucleopore polycarbonate filters for the containment of fly ash to be exposed to transplanted trachea. These fly ash packets were incubated in horse serum in vitro in order to simulate in vivo exposure. The mutagenicity of the serum was measured after 1 hour and 1, 7, and 14 days with Salmonella typhimurium strain TA-1538. The revertants per milligram were 14, 29, 36, and 51, respectively. The fly ash packets removed from the horse serum after 0, 1, 7, and 14 days were extracted with DMSO and the specific activity was found to be 53, 39, 31, and 26 revertants per milligram, respectively. These results indicated about one third of the mutagenic activity was released rapidly (1 day) and most of the remaining activity released slowly over the next two weeks. For comparison DMSO extracts of additional fly ash packets will be tested for mutagenic activity after insertion in rat tracheal transplants. We feel these techniques may be useful in the study of carcinogenesis by mutagenic particles.

Ca-12
MUTAGENICITY AND CARCINOGENICITY OF NITROSAMIDES. W. Lijinsky & A. W. Andrews, Chemical Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland 21701.

Nitrosamides are assumed to be direct-acting mutagens and carcinogens. Several of them are possible risks to man because they occur in the environment or can be formed in vivo from amides and nitrite. A comparison of their mutagenic and carcinogenic activities might provide increased understanding of the relationship between mutagenesis and carcinogenesis. More than 30 nitrosamides, including nitrosoalkylureas, nitrosoalkylguanidines and nitrosoalkylcarbamates, were prepared and tested for carcinogenicity by painting on mouse skin. Each was tested as a mutagen in Salmonella typhimurium (TA 1535). Carcinogenicity tests were at equimolar doses for 50 weeks (total 0.1 millimoles). Among the nitrosoalkylureas, nitrosomethylurea was the most effective carcinogen, but far from the most potent mutagen. Nitrosoethylurea was much less mutagenic, but only a little less carcinogenic. Several substituted ethylnitrosoureas were much more mutagenic than the parent compound, but all were less carcinogenic, excepting fluorooethylnitrosourea. Phenylethylnitrosourea was a potent mutagen, but was not carcinogenic in mouse skin. The most potent mutagen was nitrosocarbaryl, but this was a very weak carcinogen by skin painting. It is possible that the discrepancies are due to the need for activation of these compounds for carcinogenesis in mouse skin.

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Ca-13

MUTAGENICITY AND DNA DAMAGING EFFECTS OF STRONG METHYLATING AGENTS IN SALMONELLA TYPHIMURIUM. K. A. Rochefort and J. E. Cummins, Department of Plant Sciences, University of Western Ontario, London, Ontario, N6A 5B7.

Strong methylating agents are being used extensively in organic synthesis because they have unique abilities in bringing about hydrocarbon transformations. Previously we reported that methyl fluorosulphonate (Magic Methyl) was a substitution mutagen (Rochefort, Murray and Cummins, 1979). We have extended those observations by studying DNA damage in Salmonella and human leucocytes following Magic Methyl treatment. Furthermore, we have determined that Magic Methyl reacts with dimethylsulphoxide to produce a weak frameshift mutagen, dimethyl methoxysulphonium fluorosulphate and non-mutagenic trimethyloxysulphonium fluorosulphate. We have determined that two other compounds, methyl trifluoromethane sulphonate (Methyl Triflate) and trimethyl oxonium hexachloroantimonate are active as substitution mutagens. Treatment of Salmonella cells with Magic Methyl or Methyl Triflate followed by alkaline agarose gel electrophoresis shows that single DNA strands are reduced to fragments about 1000 nucleotides long. Half the DNA of treated bacteria is rapidly destroyed while the other half is stable. DNA replication is changed from an exponential to a linear pattern at a reduced rate in the treated cells. These strong methylating agents are potent DNA damaging agents and substitution mutagens. We thank Professor J. F. King, University of Western Ontario for advice and for providing superacid methylesters. The work was supported by the Canadian National Research Council.


Cb-1


Streptonigrin (NSC-45383), a direct acting clastogen, was administered intravenously to female rabbits to determine whether various cell types are differentially sensitive to aberration induction by direct acting agents. Blood was collected by cardiac puncture 30 minutes after injection and used for lymphocyte cultures. The animals were then injected with colchicine and sacrificed 3-1/2 hours later. Marrow cells from a femur and lymphoblasts from mesenteric nodes were prepared for cytogenetic evaluation. Lymphocytes from the cultures were prepared for cytogenetic examination 48 hours after PHA stimulation. Samples were evaluated microscopically for structural cytogenetic abnormalities (excluding gaps). Detailed examinations of a total of 7,000 metaphases summarized by animal and cell type then served as a data base for statistical analyses. For inclusion, samples had to contain at least 100 evaluated metaphases.

First the findings were evaluated for components of variance and dose response relationships by assuming a binomial model with cells classified as either with or without structural aberrations. Further analysis included enumeration of chromatid breaks necessary to cause the observed abnormalities and evaluation of probability density distributions as descriptors of the breakage dispersion observed in cells. Distributions evaluated were: geometric (GD), Poisson (PD), negative binomial (NBD), and the generalized Poisson (GPD). The findings from these evaluations all indicate that lymphoblasts are more sensitive to aberration induction by streptonigrin than marrow cells. (Supported by U. S. Department of Energy and NICHHD Grant HD-08828.)

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PARTITIONING OF EXPOSURE-RESPONSE CURVE INTO EXPOSURE-DOSE CURVE AND DOSE-RESPONSE CURVE. W.R. Lee, Louisiana State University, Baton Rouge, LA

The relation of exposure to dose was determined for ethylene dibromide (EDB) with exposure measured as concentration in a static exposure chamber and dose as binding of alkyl groups to DNA (alkylations/nucleotide - AN) in Drosophila melanogaster spermatids. For the exposure-dose curve there was a significant departure from linearity with a fit to an exponential curve with an exponent of 0.4. Similar shaped exposure-dose curves have been reported for EMS and NMS. The dose-response curve was determined with dose (AN) plotted against the relative frequency of sex-linked recessive lethals (slrl) determined for three dose levels. The data fit a one-hit model that extrapolates to the origin with a slope of 6 (%slrl=6 Dose AN). The shape of the exposure-dose curve is different from the dose-response curve for all three chemicals, EDB, EMS and NMS. Therefore, if a genetic interpretation had been made from the exposure-response curve the conclusion would have been incorrect. By partitioning the exposure-response curve into the exposure-dose curve and the dose-response curve we separate the physiological factors from the mutation induction response. Exposure-dose curves can be determined for species selected for a similar physiology to man and without the capability of genetic tests for mutations, while the dose-response curve can be determined in species selected for the capability for genetic tests for mutations.

EVALUATING STATISTICAL ANALYSES OF MICROBIAL MUTAGENICITY ASSAYS


The NCI has completed the first phase of a four laboratory study on the reproducibility of testing chemicals for mutagenicity in the Salmonella microsome assay. This paper is a report on the statistical analysis of some of that data. This statistical analysis involves 1) identifying and removing spurious data; 2) determining the adequacy of the remaining data in making a decision on the mutagenicity of the test data; 3) performing statistical tests; and 4) interpreting the results. Using this procedure, seven approaches to determining the mutagenicity of a test are presented. These decision rules are 1) two-fold rule, 2) modified two-fold rule, 3) one way analysis of variance, 4) test for linear trend, 5) combination of 3 and 4, 6) 97.5th percentile threshold rule and 7) the confidence interval rule. The conclusions drawn by each rule are compared to the microbiologists' interpretation, and the results of these comparisons will be presented. Finally, the strengths and weaknesses of each rule will be discussed.

A TWO-LESION HYPOTHESIS FOR MUTATION INDUCTION IN HAEMOPHILUS INFLUENZAE BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE. R. F. Kimball, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.
Previous evidence suggested that treatment of Haemophilus influenzae with monofunctional alkylating agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) causes gaps to be left in the DNA synthesized after treatment. Evidence will be presented that the gaps increase linearly with concentration of MNNG and that the lesions giving rise to them disappear, probably by repair, at about the same rate as those giving rise to mutations. Alkylated bases would also be expected to increase linearly with exposure. It will be shown that mutations, on the other hand, increase approximately as the square of the exposure. It is suggested that mutations produced by MNNG and probably by other monofunctional alkylating agents arise by a two-step process. During replication, incorrect bases (single-strand base substitutions) are inserted opposite alkylated bases, and non-coding lesions cause gaps to be left in the newly synthesized DNA. Recombination repair of gaps then converts some of the single-strand substitutions into double-strand ones. Mismatch repair shortly after replication removes most of the single-strand substitutions leaving the double-strand ones as the main source of mutations. This hypothesis also explains earlier observations on mutation fixation as measured by transformation. (Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.)

Cb-5

GENETIC FACTORS THAT AFFECT THE SPONTANEOUS RATE OF MUTATION. R. C. Woodruff and J. N. Thompson, Jr.*, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403 and Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019.

Genetic factors that alter the spontaneous rate of mutation may affect the interpretation of mutagenic screens. For example, mutator activity associated with the common male recombination (MR) chromosomes in Drosophila melanogaster appears to be suppressed in natural population lines. Crosses between natural population lines and laboratory stocks, or between geographically separated populations, however, lead to the release of mutator activity. We have observed a two to twenty fold increase in the frequency of spontaneous recessive sex-linked visible mutations after hybridization between natural population lines. In addition, the OK1 mutator line has a fourteen fold increase (from 0.20% to 2.80%) in the frequency of recessive sex-linked lethal mutations after hybridization with laboratory stocks. The frequency of mutations continues to be high in the OK1 hybrid males after fifteen generations. These results suggest that spontaneous mutation rates can be affected by genetic factors that are common in D. melanogaster, and that these factors must be considered in selecting wild-type lines for mutagenicity tests.

Cb-6

PRELIMINARY CHARACTERIZATIONS OF THYMIDINE KINASE ISOZYMES IN L5178Y MOUSE LYMPHOMA CELL LINES: K. A. Palmer and P. Voytek, Food and Drug Administration and Environmental Protection Agency, Washington, D.C.

The L5178Y mouse lymphoma heterozygous forward mutational assay originally introduced by Clive and Flamm shows promise as a predictor of potential chemical mutagens. The assay has not escaped criticism concerning the biochemical and genetic basis of the thymidine kinase locus. The present study was initiated to investigate some of the physical-chemical properties of thymidine kinase isoforms isolated from the three suggested phenotypes i.e., TK*/+, TK*/- and TK*/-. Separation and isolation of thymidine kinase isoforms present in L5178Y cells was performed by a preparative electrofocusing granulated gel technique. Km values for TdR and BUdR were determined for fractions showing thymidine kinase activity. The effect of
two thymidine kinase effectors, thymidine triphosphate (inhibitor) and deoxycytidine diphosphate (activator) were determined. Data from these experiments demonstrate certain similarities in the enzyme profile of the three proposed phenotypes. There are two pH regions where thymidine kinase activity is detected, one in the acid range (pH 6) and the second in the basic range (pH 8.5). Km values for the natural substrate TdR and the selective agent BUdR are the same for the various isozymes evaluated. Effector response indicates that differences between the various cells lines is observed for TIP but no effect is evident for dCDP. These data tend to indicate that if changes have occurred in the gene or genes they are not manifested in the active site of the enzyme but in the allosteric site. These studies, however, are preliminary and do not provide the total picture of the thymidine kinase enzyme in L5178Y cells.

Ch-7

DOSE-RELATED CYTOTOXICITY, TRIFLUOROTHYMIDINE-RESISTANCE, AND COLONY SIZE OVER TIME IN METHYL METHANE SULFONATE-TREATED L5178Y TK+/− CELLS.

D. E. Amacher, S. C. Paillet, and V. A. Ray, Safety Evaluation Department, Pfizer Central Research, Groton, CT 06340

Mouse lymphoma L5178Y TK+/− cells were treated for 3 hours with 0.2–2.0×10−M concentrations of methyl methanesulfonate (MMS) in a series of separate experiments, the mutagen removed, then allowed expression periods of either 0, 48, 96, 144 or 240 hrs. Treated and control cells were then cloned in soft-agar media (RPMI-1640+10% horse serum) for seven days using 4µg/ml trifluorothymidine (TFT) as the mutant selective agent. Interphase death measured as trypan blue exclusion was slightly enhanced on days 3–4 in MMS-treated cells. Treated cells cloned at 0 hours expression showed a dose-related decrease in average colony size in the absence of TFT. Increases in mutation frequencies were dose-related and consistent over time in MMS-treated cells when expression time varied from 48 to 240 hours and only large, TFT-resistant colonies were counted. Microscopic measurements of actual colony size showed that both MMS-treated and solvent control colony populations in the TFT-treated plates were essentially bimodal. When seven individual small colonies from TFT-treated plates were removed and grown out as clonal lines for 7 or 30 days in non-selective media, only one remained uniformly small; all others reverted to large or mixed (bimodal) populations. Further experiments with another 20 small colony-derived clones from control or MMS-treated cells demonstrated that cell stock originating from MMS-treated, large, TFT-resistant colonies were also BUdR-resistant and were THMG-sensitive. These results suggest that: (1) small colony size itself is not a stable, heritable trait, and (2) large TFT-resistant colonies represent true, stable thymidine kinase deficient mutants.

Ch-8


In our continuing effort to understand the molecular mechanisms involved in mutagenesis and carcinogenesis, we have been studying the cell cycle specificity of mutagenesis. For this purpose, we have developed a non-toxic growth quiescence synchronization technique involving serum deprivation and have reported that replicating cells are more sensitive to chemical mutagenesis by MMS (Cancer Lett. 4:277, 1978). We have further reported that this sensitivity is S-phase related (Fed. Proc. 37: 750, 1978). Because of the significance of this observation, we have proceeded to confirm it with a second synchronization procedure involving the metabolic inhibitor thymidine and a second carcinogen. Exposure of
cells synchronized by the two procedures to a 30 min. pulse of 5×10^{-5}M MMNG resulted in the highest 6-thioguanine-resistant (TG^R) mutant incidences when the exposure was during S-phase.

<table>
<thead>
<tr>
<th>Synchronization</th>
<th>G_1</th>
<th>S</th>
<th>G_2</th>
<th>M</th>
</tr>
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<tr>
<td>Serum Deprivation</td>
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<td>695</td>
<td>305</td>
<td>---</td>
</tr>
<tr>
<td>Double Thymidine Block</td>
<td>22</td>
<td>470</td>
<td>274</td>
<td>240</td>
</tr>
</tbody>
</table>

*Sporontaneous Mutant Incidences Range From 0 to 10

Thus, we believe we have an in vitro epithelial cell system in which a sensitive phase of the process of chemical mutagenesis has been defined and can be subjected to further analysis.

Cb-9

THE INFLUENCE OF AGE ON THE ACTIVATION AND METABOLISM OF AFLATOXIN B_1 BY RAT LIVER. Andrew Jayaraj#, Tom Diller, and Arlan Richardson, Illinois State University, Normal, IL 61761.

The ability of the liver from 5- to 30-month old male Fischer F344 rats to activate and metabolize aflatoxin B_1 (AFB_1) was studied. Using the Salmonella/microsome test, the conversion of AFB_1 to mutagenic metabolites by liver homogenates (S-9 fractions) from rats of various ages was compared. The S-9 fraction from the liver of 12-month old rats produced 2-fold more revertants than the S-9 fractions from either 5-, 18-, or 27-month old rats. There was no significant difference in the ability of the S-9 fractions from liver of 5-, 18-, and 27-month old rats to activate AFB_1. Liver microsomes from 12-month old rats also had a greater ability to activate AFB_1 than microsomes from either 5-, 18-, or 27-month old rats. The ability of S-9 fractions from the liver of 5-, 12-, and 20-month old rats to form [^{3}H] AFB_1-DNA adducts was determined also. The S-9 fraction from 12-month old rats formed approximately 2-fold more AFB_1-DNA adducts than the S-9 fractions from either 5- or 20-month old rats. The conversion of [^{3}H] AFB_1 to water- and chloroform-soluble metabolites by S-9 fractions isolated from the liver of 5-, 12-, and 20 month old rats was measured using thin layer chromatography. Most of the chloroform-soluble radioactivity migrated as AFQ_1. An age-related difference in both the amount and proportion of AFB_1 metabolites formed was observed. The metabolism and activation of AFB_1 by the liver does change significantly with increasing age. The age-related decrease in AFB_1 activation after 12 months of age could be related to the occurrence of testicular tumors, which has been observed in this strain of rats. (Supported in part by NIH grant RO1 CA24856).

Cb-10


The induction and expression of 6-thioguanine resistant mutants in CHO cells which were maintained in a non-dividing viable state in serum free medium were studied with the mutagen ethyl methanesulfonate. The re-addition of serum containing medium at different times post mutagen treatment allows modulation of the time interval before DNA replication and cell division occurs, in these studies 0-14 days post treatment. As compared to logarithmic phase cultures, similar mutant frequencies were found with cultures to which serum containing medium was added immediately after mutagen treatment, and an identical phenotypic expression time of 7-9 days. Maintenance of cultures for up to 14 days post treatment in the arrested state resulted in no decline in the mutant
frequencies, but shorter expression times were observed. These results are consistent with the proposal that the mutagenicity of EMS does not require normal DNA replication for mutation fixation. In addition, the mutagenic damage does not appear to be "repaired" in these arrested cell cultures.

(Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.)

Cb-11

The CHO/HGPRT mutational system has been useful to quantify the mutagenic effects of various chemical or physical agents singly. Demonstration of the ability to determine the interactive effect between physical and chemical agents would enhance the utility of this system in environmental mutagenesis. Due to the prevailing use of 8-MOP for treatment of psoriasis and vitiligo, we have studied the interaction of 8-MOP and near UV light on the cloning efficiency and mutation induction to 6-thioguanine resistance under standard conditions. Concentrations of 8-MOP up to 20 μg/ml or fluences of near UV up to 40,000 J/m² when administered individually have no effect on survival or mutation frequency. To assure incorporation of the 8-MOP, cells were incubated with the photosensitizer prior to irradiation for 5 to 120 min. Under all experimental conditions, mutation frequency was not influenced by the length of time allowed for incorporation of the chemical. Cellular proliferation, as measured by cell number 20 hrs after treatment, was 20% of the control when cells treated with 20 μg/ml 8-MOP were exposed to a near UV fluence of 300 J/m². A similar reduction of survival was also observed. Mutation frequency increased linearly until the survival fell below 20-30% when either the 8-MOP concentration or the near UV fluence was increased while the other factor was held as a constant. Mutation induction by the interaction of 8-MOP and near UV light, thus, appears to be quantifiable.

(Research sponsored jointly by the Environmental Protection Agency under Interagency Agreement DE-ES81-A0 and by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.)

Cb-12

Aphidicolin is a specific inhibitor of DNA polymerase α and blocks DNA synthesis in vivo. The inhibition has been shown to be reversible with excess dCTP but not with the other three deoxynucleoside triphosphates. In order to study the various roles that the α-polymerase might play in DNA replication and/or repair, we have attempted to isolate Chinese hamster V79 and CHO cells that are resistant to aphidicolin. Resistant mutants were isolated from both cell lines mutagenized with either BrdU-black light and UV (V79) or with EMS (CHO). None of the aphidicolin-resistant mutants so far examined contains an α-polymerase that is resistant, in crude extract measurements, to aphidicolin. Furthermore, the specific enzymatic activities for the α-polymerase in crude extracts of the mutants are the same as those for the V79 and CHO wild type cells. One of the V79 cell mutants, aphl-4 which was characterized in greater detail, appears to be defective in pyrimidine biosynthesis. Comparative studies
of this mutant with the V79 wild type cells revealed the following: (1) aph⁻⁴ was significantly more UV-sensitive but not more X-ray sensitive; (2) following the incorporation of BrdU, aph⁻⁴ exhibited a nine-fold higher frequency of chromatid gaps and breaks; (3) UV-induced mutation frequencies in aph⁻⁴ at the ouabain-, 6-thioguanine-and diphtheria toxin-resistant loci were significantly higher than those for the wild type. Additional experiments to further characterize this mutant and to identify its biochemical lesion are underway. Research supported in part by grants from NCI (CA21104) to J.E.T., from NIAID (AI 14357) to J.A.B. and from NIH (ES01809) to C.C.C.

Cb-13
CAUSES OF STERILITY IN MALE MICE DERIVED FROM GERM CELLS TREATED IN SPERMATOGONIAL OR POSTSPERMATOGONIAL STAGES WITH CYCLOPHOSPHAMIDE.

Nester L.A. Cachefro and Ethel L. Russell, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

In previous studies we have found that the incidence of chromosome aberrations associated with male sterility in the progeny derived from x-ray or chemically-exposed spermatogonia is much lower than that in sterile sons derived from treated post spermatogonial stages. We have now analyzed sterile males recovered in cyclophosphamide experiments of W. M. Generoso, in which various stages were treated. A total of 42 F₁ sterile males were studied cytologically and histologically. Chromosome aberrations were detected in 2 of 4 sterile males derived from treated spermatogonial stages, one male being XXY and the other XX; T(Y;17). Both males had small testes and spermatogenic arrest. Chromosome abnormalities were found in 31 out of 38 F₁ sterile males derived from treated post spermatogonial stages. In this group, all but one (inversion in chromosome 1), were carriers of reciprocal translocations, 12 involving the Y chromosome. Among the 30 translocation carriers were 4 carrying two translocations each, two with a tandem translocation involving 3 chromosomes, and one also carrying an inversion. In all cases, at least one of the translocation breakpoints was near the end of a chromosome, except when the Y chromosome was involved. To date, all X-autosome translocations in the mouse have been male sterile, which explains the high incidence of this type of translocations in the sterile group. Sterile F₁ males produced from treatment of spermatogonial stages are rarely translocation carriers. If they show chromosome anomalies, these are most commonly the result of sex-chromosome nondisjunction. [Research sponsored by the Dept. of Energy under contract with Union Carbide Corp.]

Cc-
SISTER CHROMATID EXCHANGES IN HUMAN LYMPHOCYTES AFTER G₀ EXPOSURE TO MITOMYCIN C (MMC): EFFECTS OF CONCENTRATION AND EXPOSURE TIME

L. G. Littlefield, S. P. Colyer*, and R. J. DuPrain, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN 37830

Studies have demonstrated that SCEs show a linear dependence on dose and exposure time in human lymphocytes exposed to MMC prior to culture. To define the inter-relationships of MMC concentration and time of exposure on SCE induction, G₀ lymphocytes from a woman were exposed to 2.5 µg/ml MMC for 2 h, washed and cultured with equal numbers of untreated male lymphocytes. SCE frequencies were within normal range in cultures containing untreated female or male cells, or equal numbers of each. In cultures containing MMC-treated female cells, a mean of 119 SCE/metaphase was observed in X-bearing metaphases, while SCEs were not elevated in Y-bearing cells. This verifies that SCE induction following G₀ exposure is not the result of carry-over or release of MMC into the culture. Subsequently, G₀ lymphocytes were exposed to 0.5 or 1.0 µg/ml MMC for 5 min to 3 h, and to 0.25 or 0.5 µg/ml for 3-24 h. Increased SCEs were observed
after G0 exposures for as little as 15 m to doses of 1.0 µg/ml MMC. At all doses, SCE induction was linear over the time intervals tested. To compare SCE induction between cultures, we calculated SCEs/µg/h for these cultures and for an additional 20 cultures exposed to doses ranging from 0.25 to 2.5 µg/ml MMC for 2 h. A mean frequency of 20.4 ± 5.0 SCEs/µg/h was calculated for the 32 cultures. These findings suggest that a single term adequately describes SCE induction after exposures of G0 lymphocytes to 0.25 - 2.5 µg/ml MMC for time intervals of 30 min to 18 h. (Supported by USDOE contract no. DE-AC05-76OR00033 and NTH Grant HD08828.)

Cc-2
EFFECT OF SODIUM AZIDE ON SCE'S IN HUMAN LYMPHOCYTES AND CHINESE HAMSTER CELLS. P. Arenaz and R. A. Milan, Washington State University, Pullman, WA 99164.

Previous results from this laboratory indicate that sodium azide, specifically its metabolite, is a unique mutagen. It is highly mutagenic in the S. typhimurium base substitution line TA1530, but not in the frame-shift tester lines, as well as in barley, peas, rice, and Chinese hamster cells. On the other hand, azide apparently does not produce chromosome breaks in barley and human lymphocytes. The latter results suggested a study of the effects of azide and its metabolite on sister chromatid exchanges.—Human whole blood and Chinese hamster K-1 cell cultures were exposed for 4 h to concentrations of azide ranging from 10-3M to 10-7M followed by BrdU for 48 h. Cells were harvested and the FPG technique was utilized. Concentrations of azide above 10-4M produced lethality in both lymphocyte and Chinese hamster cultures. The lower concentrations of azide produced no significant increase in SCE frequency above background (5-7 SCE's/cell and 10-11 SCE's/cell for lymphocytes and Chinese hamster cells, respectively). Azide metabolite was added to cultures of both lymphocytes and K-1 cell line at various concentrations and times. Again, there was no significant increase in the frequency of SCE's above control level. Mitomycin C treatments run concurrently produced very significant increases in SCE's.—This apparent lack of induction of SCE's above control and previous data on the negligible clastogenic potential of azide confirms the uniqueness of this mutagen. It would appear that azide is one of the few known mutagens that do not increase SCE's and/or produce chromosome breaks. This data lends credence to the postulate that azide and its metabolite are specific for base substitution. (Supported by DOE Cont. #EY-76-S-06-2221.)

Cc-3
SISTER CHROMATID EXCHANGE STUDIES IN PETROLEUM REFINERY WORKERS. A.V. Carrano, L.B. Harrison*, B.H. Mayall*, J.L. Minkler* and F. Cohen*, Biomedical Division, Lawrence Livermore Laboratory, Livermore, CA, University of Pennsylvania, Philadelphia, PA and Newark Beth Israel Hospital, Newark, NJ.

Individuals are exposed daily to a wide variety of genotoxic chemicals and so there exists the need both to identify these chemicals and to estimate the potential somatic or genetic damage to man. The sister chromatid exchange (SCE) assay is one method to assess such damage. It is directly applicable to the human lymphocyte, it quantifies a lesion which, for several chemicals, bears a close relation to the mutagenic lesion, and it has demonstrated sensitivity as an indicator of in vitro and in vivo exposure. For these reasons, we are measuring the lymphocyte SCE frequency of individuals that are: 1) "non-exposed"; 2) cancer patients; or 3) workers employed in the petroleum refinery industry for at least ten years. For eighteen "non-exposed" individuals the mean SCE frequency was 0.19 ± 0.02 (S.E.) SCE per chromosome. Of the cancer patients, four were not undergoing
chemotherapy and only one possessed an elevated SCE frequency. The ten additional patients undergoing maintenance chemotherapy for a variety of cancers served as positive controls. Eight of these showed a significantly elevated SCE frequency. Of twenty-two workers in the petroleum refinery industry about half had SCE frequencies which were more than two standard errors above the "non-exposed" group mean. Preliminary analysis of the personal health and work histories suggests that neither smoking nor medication can account for the increases and that they may reflect occupational or other environmental exposure. (Supported by U.S. DOE contract W-7405-ENG-48.)

Cc-4

CYTOGENETIC ANALYSIS OF INDIVIDUALS WITH AN INHERITED PREDISPOSITION FOR CANCER. R. G. Moon, E. J. Gardner, and J. P. Hughes. University of Utah Research Institute, Salt Lake City, UT 84108.

Chromosome anomalies have been demonstrated in a variety of tumors and cancerous tissues. Sister chromatid exchange frequencies have also been examined in a number of neoplastic tissues and certain human genetic diseases. Individuals with inherited predisposition to colorectal cancer, i.e. familial polyposis coli and Gardner syndrome, comprise the study group. Chromosome examination of skin, polyfibreplasts and peripheral blood lymphocytes were analyzed with g-banding and sister chromatid exchange (SCE) analysis. Although SCE frequencies were not significantly different than the controls (5.8 SCE/diploid metaphase), increased aneuploidy was observed in the affected individuals (47-180 chromosomes/metaphase). In addition, we are currently examining fibroblast cultures following exposure to known mutagens to evaluate possible cytogenetic markers and also evidence of a change in sensitivity of the treated chromosomes as reflected by altered SCE frequency. This study is designed to determine whether a detectable cytogenetic marker is associated with familial colon cancer and would thus aid in early identification of individuals at risk for the disease.

Cd-7


The sensitive-indicator (SI) method is based on our earlier finding that a few specific, easily observed, anomalies are indicators of about one-fourth of all serious dominant skeletal mutations. Coded skeletons of progeny sired by treated and control males are examined for a few specific malformations, these being the SI's. Mice with SI's are thought likely to be mutants because in our earlier work mice with any of these effects were shown to be mutants by breeding tests. Other presumed dominant skeletal mutations, based on presumed-mutation criteria (Selby and Selby, 1978, Mutat. Res., 50: 341-351) and here called PM's, can also be detected when doing these examinations. Various radiation and chemical treatments of spermatogonia are being used in an attempt to determine the efficiency of this method and whether the relative mutation frequencies for dominant mutations under different conditions parallel those found using the specific-locus method. To date, the frequency of these events is 2/110 (1 SI + 1 PM) for males exposed to 100 R + 500 R of 93 R/min X radiation (24 hrs between fractions); 2/184 (1 SI + 1 PM) for males exposed to 600 R of 93 R/min X radiation; and 0/472 for control males. The frequencies of these events in the fractionation experiment alone, and in the two irradiation experiments combined, are significantly higher than the control frequency (P = 0.04 and P = 0.02 in Fisher's exact test, respectively). Early results are thus most encouraging. Should the control frequency prove to be low enough to make the SI method highly efficient, this proce-
dure will have great potential as a screening method in chemical mutagenesis testing, especially because it is based on the type of data easily made use of in risk estimation (Selby and Selby, 1977, Mutat. Res. 43: 357-375). [Research sponsored by Dept. of Energy under contract with Union Carbide Corp.]

Cd-2
DETECTION OF SOMATIC MUTATIONS IN HUMAN ERYTHROCYTES--INITIAL HEMOGLOBIN RESULTS AND DEVELOPMENT OF GLYCOPHORIN A AS A NEW MARKER. W. L. Bigbee; Biomedical Sciences Division, Lawrence Livermore Laboratory, Livermore, California 94550

With the goal of being able to detect the effects of genotoxic agents in man, we are developing methods to measure somatic mutations in individual human cells. Initial efforts have been directed at determining the frequency of hemoglobin S- and C-containing erythrocytes in individuals who have genetically normal hemoglobin A. This is done by labelling these rare cells with fluorescent antibodies made monospecific for these single amino acid substitutions. The cells are then enumerated using a high speed cell sorter. Initial results indicate a background frequency in 6 normal individuals of $10^{-8}$ to $10^{-7}$ for both events. The significance and genetic validation of these results will be discussed. Recent work has focused on an additional marker available in erythrocytes. Glycophorin A is an externally presented membrane antigen carrying the MN blood group determinants. Experiments will be outlined to show how point mutation and gene deletion type mutation frequencies can be determined for this protein. Work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore Laboratory under contract number W-7405-ENG-48 with the support of DOE/NTEHS Interagency Agreement 222-Y01-ES-70031 and NIEHS contract No. N01-ES-9-0002.

Cd-3
VARIATION IN THE LEVEL OF $\alpha$-GLYCEROLPHOSPHATE DEHYDROGENASE IN SINGLE SPERM AFTER TREATMENT WITH PROCARBAZINE, H. V. Melling, C. P. Ray, and J. G. Burkhart, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

The mitochondria of mouse sperm contain $\alpha$-glycerolphosphate dehydrogenase. The enzyme is located between the outer and inner membrane of the mitochondria. A specific histochemical stain was developed for this enzyme using nitro blue tetrazolium, and presence of the enzyme was indicated by accumulation of the purple formazan. The amount of formazan was measured using an Axiomat microscope and photometer. The accumulation of formazan was linear with time up to 30 min at 37°C. The amount of formazan accumulated during the first 30 min of the process is likely to be proportional to enzyme activity. DBA/2J male 10-12 weeks old were treated with various doses of procarbazine for 100 to 800 mg/kg. The content of $\alpha$-glycerol-phosphate dehydrogenase was measured in individual sperm from mice killed at 4-day intervals up to 50 days after the injection of procarbazine. In the concurrent control the enzyme levels were constant during the total screening period, and of 4200 sperm from control only two did not have any stain at all. The frequency of stained sperm varied with dose of procarbazine and the time after treatment. No effect, however, was seen after a dose of 100 mg/kg. After treatment with 200 mg/kg, the frequency of non-stained sperm is elevated in the samples taken 43 and 48 days after injection of the mutagen. The frequency of unstained sperm increased with increasing dose. The time interval between the injection and a significant increase in the frequency of unstained sperm shortened with increasing doses of procarbazine. The mechanism for induction of unstained sperm will be discussed.
Cd-4
SURVEY OF ESTUARINE SPECIES AS BIOACCUMULATORS OF MUTAGENS. J. R. Baylis, T. H. Sparks*, and C. W. Chang*, University of West Florida, Pensacola, FL 32504.

Commercially obtained shrimp (Penaeus sp.) and oysters (Crassostrea virginica) were examined for detectable levels of mutagens. Solvent partitioning of whole tissue homogenates was employed, using isopropanol, acetone, and chloroform extractions followed with concentration by flash evaporation. The extracts were processed through an XAD-2 column to eliminate histidine. Extracts were assayed with and without Aroclor-induced rat liver activation mixture in the Salmonella/microsome mutagenicity test using strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538. Positive results were obtained with shrimp extracts from suspected pollution sites, while oyster extracts from the same site yielded negative results. Identification of the responsible compound(s) is being pursued. Other estuarine organisms are under investigation.

(Supported by a grant from the Environmental Protection Agency.)

Cd-5
CHEMICAL MUTAGENESIS IN THE NEMATODE, CAENORHABDITIS ELEGANS. K.K. Lew, D. Bilodeau*, C. Harding* and E. Zentz*, Children's Hospital Medical Center and Department of Neuropathology, Harvard Medical School, Boston, MA 02115.

An in vivo mutagenic assay has been developed in Caenorhabditis elegans, a free-living soil nematode. The assay takes advantage of three unique properties of this animal: 1) a large population of animals can be grown for mutagenic assay (≥ 500,000), 2) the animal has a short generation time (3 days at 25°C), 3) homozygous recessive mutants can be readily generated (the animal is normally hermaphroditic). We have isolated a series of mutant strains which are small in size relative to wild type and we have used these mutants as genetic markers to detect mutagenesis. The assay is the reversion of small animals to large wild-type animals. Revertants can be readily counted because of the great difference in size. A filtering system which can detect rare revertants in a population of 500,000 has been developed. Mutagenized small animals are allowed to swim through a uniform nylon mesh; revertants—which are much larger than mutants—are trapped on top of the filter and are subsequently counted. Known mutants benzo (α) pyrene, 2-anthramine, MMS, EMS, and acridine orange, as well as a blind testing of chemicals from the Research Triangle Institute were tested. Our assay system can detect microgram quantities of chemicals and, so far, the test with this animal does not require the addition of S-9 preparations to activate chemicals. The assay of some S-9 microsomal enzymes in C. elegans will also be discussed. This work was supported by N.I.H. grant S ROI AG 00971-02.

Cd-6

The use of standard microbiological assay methods for quantifying the mutagenicity of volatile substances has met with mixed success. In many instances, mere identification of the mutagenicity of volatile substances is difficult. For example, the potent carcinogen, vinyl chloride, exhibits very weak apparent mutagenicity when tested with
the Ames/Salmonella his\textsuperscript{+} reverse mutation assay. We ask whether such substances are actually weak mutagens, or does their volatile nature prevent proper assay with the bacterial test? The efficient identification and quantification of vapor phase mutagens is of considerable interest since ambient air, for example, is presented with $5 \times 10^{11}$ g/day of vapor-phase pollutants, as compared with $4 \times 10^{11}$ g/day of industrially-produced particulate matter. We report modifications of the Salmonella bacterial mutagenesis assay which enable us to quantify the mutagenicity of volatile substances, such as ethylene oxide, styrene oxide, etc. Manipulations include the use of cells in the log phase (vs. stationary phase) of growth, preincubation in liquid-suspension, use of sealed vials and soft agar. The volatile substance is contained within the culture dish by sealing the plate. The degree of increased sensitivity of detection of volatile mutagens afforded by each manipulation, and the applicability of the method to the detection of direct-acting and promutagens will be discussed. Supported by U.S. EPA Contract 68-02-2724.

Cd-7
QUANTITATION OF MAMMALIAN CELL RECOVERIES IN THE PERITONEAL HOST-MEDIATED ASSAY. James W. Barnett, Jr., and Jonathan B. Ward, Jr. University of Texas Medical Branch, Galveston, Texas 77550.

Measures of recovery and survival of indicator mammalian cells introduced into the peritoneal cavity of mice have been confounded by the presence of endogenous lymphocytes and macrophages in the initial recovered fluids. Multi-channel analysis of particle size determinations on the Coulter model ZBI revealed that two major distinct cell populations were being removed in our host-mediated assay with Balb/c 3T3 cells. Balb/c mice inoculated with homogenized cell membranes demonstrated only the single population of smaller particle size. By utilizing appropriate instrument window settings, we have been able to differentiate and quantitate immediately the indicator and endogenous populations in recovery fluids. Typical 3T3 cell recoveries range from 5-20% of initial inoculum. Recovery fluids contain between $6 \times 10^5$ - $3 \times 10^6$ macrophages/ml of which 60 - 80% are rinsed away at four hours. The remainder attach firmly to the culture substrate and appear less sensitive than the 3T3 population to trypsinization at 24 hours. Cultures were sampled at 4 hours to determine the # of non-attached or non-viable BALB/3T3 cells. Cells removed from DMSO solvent treated mice had the lowest percentage of non-attached cells, while pyrene and benzo(A)pyrene produced increased fractions of floating cells in a dose related response. Despite some variability in initial cell recovery, cells from control animals demonstrated similar growth kinetics with approximately a four-fold increase in 48 hrs. Host-mediated treatment with cyclophosphamide showed a reduced proliferative capacity of recovered population with increased dose. Quantification of cell recovery in mammalian cell host-mediated assays should permit an experimental approach for improving the efficiency and uniformity of cell recovery as well as providing data on treatment-associated cytotoxicity.

Ce-1
MEASURING SUBTLE DIFFERENCES IN HUMAN DNA REPAIR SYSTEM AND POSSIBLE CORRELATION WITH COLONY FORMING ABILITY. D.F. Minka, P. Yu* and R.M. Antley, Indiana University, Indianapolis, IN 46202.

The principle of defective DNA repair being etiologically important in the development of cancer (Xeroderma pigmentosum) suggests the possibility that subtle differences in DNA repair among humans may relate to susceptibility of malignancy. It is the long term purpose of this research to evaluate small differences in the ability of normal human fibroblast to repair DNA. Parameters have been developed with
sufficient sensitivity to distinguish subtle differences in repair. A statistically define dose response curve is generated after quantitatively measuring unscheduled DNA synthesis (Trosko and Yager). Results will be presented on the correlation of subtle repair differences with differences in colony forming ability (Robbins et al.) Preliminary results of this study provide evidence for correlation between decrease colony forming ability and decreased DNA repair as measured by UDS. Thus indicating a relationship between repair ability and biologically significant functions of growth and division. Support ed in part by PHS P50 GM 21054 and PHS T32 GM 07468.

Ce-2

REPAIR OF UV DAMAGE IN HUMAN CELLS ALSO EXPOSED TO AGENTS CAUSING CROSSLINKS, MONOADDUCTS, AND ALKYLATIONS

D. C. Gruenert and J. E. Cleaver*, Laboratory of Radiobiology, U.C.S.F., San Francisco, California

Concomitant exposure of cells to several different DNA damaging agents has previously been used to determine whether the repair of the damage of each agent proceeds by common or independent pathways. Our purpose was to undertake such a series of experiments using UV light in conjunction with psoralen derivatives or alkylating agents. Excision repair of UV damage in human cells was measured by the incorporation of new bases into DNA after exposure to UV-B light and either psoralen derivatives (8-methoxypsoralen, angelicin, 4'-aminomethyl, 4, 5', 8-trimethylpsoralen (AMT)) plus UV-A or alkylating agents (methyl methanesulfonate, dimethyl-sulfate, and N-methyl-N'-nitro-N-nitrosoguanidine). Repair-replication was measured by CsCl-CsSO₄ alkaline isopycnic gradients and unscheduled DNA synthesis by scintillation counting or autoradiography. Alkylating agents reacted strongly with UV repair and markedly reduced the amount of repair replication observed with UV-B alone. The psoralen derivatives showed no noticeable effect on repair of UV damage. Whereas previous studies have used concomitant exposures to two different agents to determine whether the damage the produce is repaired by common or diverse pathways, these results indicate that such a method is an unreliable indicator of the number of repair pathways. We consider it possible that the observed effect of alkylating agents on UV repair is due to alkylation damage to high molecular weight UV repair enzymes.

Ce-3

DNA REPAIR AND MUTAGENESIS IN UV LIGHT-HYPERSENSITIVE MUTANTS OF CHO-AT3-2 CELLS. G.M. Adair and J.M. Clarkson*, Science Park-Research Division, The University of Texas System Cancer Center, Smithville, TX. 78957.

Using nonselective isolation procedures, we have obtained a series of mutants of CHO-AT3-2 cells that are 5-7 fold more sensitive than wild-type (in terms of D50 dose) to the cytotoxic effects of 254m UV light. Complementation analysis by polyethylene glycol-mediated cell hybridization has identified four complementation classes among these isolates. Mutants from three classes show marked deficiencies in DNA repair replication and unscheduled DNA synthesis following UV-irradiation. While displaying similar hypersensitivity to UV (~7 fold more sensitive than wild-type) a mutant representing a fourth complementation class has approximately normal levels of DNA repair replication and unscheduled DNA synthesis, and appears to have a defect in postreplication repair. The parental strain from which these mutants were derived is functionally heterozygous at both the aprt and tk loci, allowing mutagenesis assay by single-step selection of autosomal recessive 8-AA' and 5-FUdR' mutant.
phenotypes as well as 6-TG\textsuperscript{R} and OUA\textsuperscript{R} mutants. Comparison of the UV mutability of wild-type and DNA repair-deficient strains indicated that the mutant strains were more sensitive than normal cells to both the mutagenic and cytotoxic effects of 254nm UV light. The observation, in some cases, of significantly higher mutation frequencies at equivalent survival suggested that these strains are more mutable per lethal event than normal cells. (Supported by grants: NIH 5 R01 CA-04484 and ACS IN-121A/RR 5511-17).

Ce-4
TOXICITY AND MUTAGENICITY OF SHALE OIL RETORT PRODUCT WATERS PHOTOCAT-
VATED BY NEAR ULTRAVIOLET LIGHT. Gary F. Struewe, and Richard J. Brake,* Genetics Group, University of California, Los Alamos Scientific Labora-
tory, Los Alamos, New Mexico 87545

The retort processes currently being developed for oil shale give rise to "product waters" that contain a wide spectrum of organic and inorganic components, including significant quantities of UV-absorbing and fluores-
cent material. We have found that photoactivation of these "waters" with near UV-light (300-400 nm) results in biologically damaging effects at both the molecular and cellular levels. The relative photoactivity of prod-
uct waters and known procarcinogens we have tested appear to correlate well with metabolic activation results reported by others. Photoactivated product waters react with DNA in solution, resulting in several kinds of DNA damage, including 1) strand breaks, 2) alkali labile sites, and 3) interstrand crosslinks. Parallel experiments with cultured human skin fibroblasts (incapable of metabolically activating compounds) demonstrate that photoactivated product waters are both cytotoxic and mutagenic (HGPRT locus), and show good quantitative correlation with the DNA damage assay. When normalized to survival, the mutation frequencies are as great as those we find for far UV light (principally 254 nm). Repair deficient fibro-
blasts from individuals with xeroderma pigmentosum (complementation Group A) show increased cytotoxicity and mutagenicity upon exposure to photo-
activated product waters, again similar to their response to far UV light. Thus, photoactivation of oil shale byproducts (e.g., by sunlight) presents a significant and hitherto unassessed environmental risk. [This work was performed under the auspices of the United States Department of Energy.]

Ce-5
COMPARISON OF THE EFFECT OF CHEMICAL MUTAGENS IN REPAIR-
DEFICIENT AND SUFFICIENT AD-3 MUTANTS OF NEUROSPORA CRASSA.
J. O. Converse,* J. D. Stewart* and T. Ong. Microbiology Section, DRDS, NIOSH, 944 Chestnut Ridge Road, Morgantown, WV 26505.

The excision repair deficient marker uvs-2 was crossed into the tester strains N23 and N24 of Neurospora crassa. Compari-
sions were made among the effects of a variety of mutagenic agents in repair sufficient strains (N23 and N24) and repair deficient strains (N23 uvs-2 and N24 uvs-2) with regard to cell killing and induction of reverse mutation from adenine dependent (ad-3) to adenine independent (ad-3\textsuperscript{R}). Methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), N-
methyl-N'-nitro-N-nitrosoguanidine (MNNNG) and ICR-170 were found to be more toxic to N23 uvs-2 and N24 uvs-2 than to N23 and N24, respectively. Both repair deficient strains are extremely sensitive to killing caused by 2,3,5,6-tetra-
ethyleneimino-1,4-benzoquinone (TEB). Excision repair de-
ficient strains appeared to be more sensitive than repair
sufficient strains to MMS, EMS, MNNG and TEB for the induction of reverse mutation. The reverse mutation frequency induced by ICR-170 in N24, however, is 20 times higher than that induced by ICR-170 in N24 uvs-2. The results reported here, in general, are similar to those found by Brockman et al., (1978, Mutation Res. 53:156) in the forward mutation assay system.

Ce-6
ROLE OF DNA REPAIR IN UV RADIATION AND CHEMICAL MUTAGENESIS IN CHO CELLS STUDIED WITH REPAIR-DEFICIENT MUTANTS. L.H. Thompson, K.W. Brookman*, and C.L. Mooney*, Biomedical Division, Lawrence Livermore Laboratory, Livermore, CA 94550.

Recently isolated UV-sensitive strains that are deficient in the repair of DNA damage were used to study the role of repair in mutagenesis. Two hypersensitive strains (UV-5 and UV-20) representing genetic complementation classes 1 and 2, respectively, were compared to wild-type (WT) cells in terms of mutation induction and survival (from 1.0 to 0.1) after treating exponential cultures with germicidal UV radiation or with 7-bromo-2-methylbenz(a)anthracene (7-BMBA). Mutations were quantified for the genetic markers thioguanine resistance (TG<sup>F</sup>) and azadene resistance (AA<sup>F</sup>) after maximal expression. WT gave nonlinear mutation induction by UV; below 50% survival, mutation frequencies were approximately constant with increasing dose. The mutant strains were 10-fold more sensitive to mutation, had linear dose responses, and yielded frequencies 2-3 times higher than WT cells. These results suggest that the repair of UV damage in WT is an error-free process that limits mutation frequencies, thus allowing toxicity to predominate. Both mutant strains were 5-fold hypersensitive to killing by 7-BMBA; initial mutagenicity results indicate a behavior qualitatively different from that after UV. Dose responses for TG<sup>F</sup> were nonlinear for WT and both mutants. WT showed continually increasing mutations with 7-BMBA dose. In contrast, the mutant strains were hypersensitive at low doses but were noticeably hypomutable at doses that reduced survival of WT. Thus, the repair process may be error-prone or may involve different efficiencies for mutagenic vs lethal lesions. Supported by U.S. DOE contract W-7405-Eng-48.

Ce-7
SYNERGISM OF EMS AND MNNG MUTAGENESIS IN EXCISION REPAIR DEFECTIVE BACTERIA AND HUMAN CELLS. R. Mandel, G. Zuerndorfer* and R.M. Baker, Boston Univ. School of Medicine and M.I.T.

The combined effect of the alkylating agents EMS and MNNG were tested using a bacterial mutagenesis assay, utilizing Salmonella typhimurium tester strains and measuring induction of resistance to 8-azaguanine, reversion of histidine auxotrophy and toxicity. Their combined effect on excision repair defective bacteria was approximately 10 times greater than expected on the basis of additivity. This synergism was observed on both mutational loci by measuring the mutational frequency (mutants/survivors) at variable dose of MNNG in the presence or absence of 5 mM EMS. Similar but smaller (2-3 fold) synergism was observed using strain TA-100 which contains a plasmid conferring extreme sensitivity on the tester strain. By contrast, repair proficient strain TA-1975 displayed approximate additivity when tested with the same mutagens. In order to test the generality of these results, the effect of EMS and MNNG was measured on repair proficient CHO cells and SV40 transformed human fibroblasts. In both cases, the dose response showed approximate additivity in the induction of thioguanine-resistance and also azadene- or ouabain-resistance. By contrast, the combined effect of these mutagens on the SV40 transformed excision repair
defective Xeroderma pigmentosum human cell line was synergistic in the induction of both thioguanine- and ouabain-resistance. Thus, there is a good correlation between the results of the bacterial and mammalian assays. The combined effect of EMS and MNNNG is synergistic in excision repair defective cells. Repair proficient cells by contrast show approximately additive effects. These results are independent of the mutational locus tested. This research was supported by the Whitaker Fund, by the NIGMS and by the NCI.

C6-8

Recent work in other laboratories has indicated that O\(^6\)-alkylguanine adducts formed in DNA by simple monofunctional alkylating agents may be important mutagenic lesions, and that such adducts may be excised in repair-proficient cells but not in human cells with the genetic defect Xeroderma pigmentosum (XP). Therefore we have compared the mutagenic responses to MNNNG and EMS in established lines of wild type (WT) and XP human fibroblasts. Cell viabilities and frequencies of thioguanine-resistant (TG-r) and ouabain-resistant (Oua-r) mutants were determined as a function of dose to the SV40-transformed lines GM637 (WT) and XP12(group A). While the XP cells were at most marginally more sensitive than the WT control cells to cytotoxicity from MNNNG treatment, induction of mutations occurred at substantially lower doses in the XP cells. Compared to the WT, about six-fold lower doses were sufficient to induce TG-r mutants at 4x10\(^{-5}\) per survivor in the XP cells. An analogous but less pronounced effect was observed with EMS. In both cases there was enhanced induction of Oua-r as well as TG-r mutants. Thus in terms of mutants induced per surviving cell by these simple alkylating agents, the XP cell line is hypermutable compared to the WT. These results are consistent with the idea that the XP cells are deficient in excision-repair of a class of alkyltion adducts that are mutagenic but not particularly cytotoxic (presumably O\(^6\)-alkylguanines). Moreover, the hypermutability of the XP cells by MNNNG and EMS can be further augmented by synergistic interaction of these agents (Mandel et al, these abstracts), and may be highly relevant to the disease syndrome. (Supported by NIGMS and NCI.)

C6-9
A COMPARATIVE EVALUATION OF MICROSPUSSION MICROBIAL DNA REPAIR SYSTEMS.

The efficacy of E. coli WP2 (uvrA\(^+\) recA\(^+\)), WP100 (uvrA\(^-\) recA\(^-\)); E. coli W3110 (polA\(^+\)), p3478 (polA\(^-\)); B. subtilis H17 (rec\(^+\)) and M45 (rec\(^-\)) to detect primary DNA damage was evaluated in a microspension assay. The six bacterial strains were exposed to multiple dilutions of twenty chemicals which were chosen to reflect the molecular diversity of direct and promutagenic agents. Preferential kill, implicative of DNA damage and revealed as an observable increase in toxicity for deficient strain(s), was demonstrated for all test agents. A comparative analysis indicated that the polA\(^+/\) strains were the least sensitive. E. coli WP100 and B. subtilis M45 were equally efficient in the detection of recombinational responsive damage. However, WP100 failed to detect the DNA modifying activity of benzo(a)pyrene. With the exception of Mitomycin C, which markedly inhibited M45, all agents eliciting excisable damage exerted enhanced selective toxicity towards the double mutant WP100 as compared to the repair deficient Bacillus strain. The two exceptions noted may be attributable to differences in cell permeability of gram positive and gram
negative organisms. The WP2/WP100 and H17/M45 microsuspension assays for DNA damage appear to be complimentary and ideally suited for the detection of agents such as natulan, NaAsO\(_2\), and Na\(_2\)HASO\(_4\) which induce DNA damage unrelated to the restoration of histidine synthesis.

**Cf-2**


Asynchronously growing cells of a V79 subline of the Chinese hamster were uv-irradiated at wavelength 254nm, using uv-illuminences up to 5.2 J/m\(^2\). Prior to irradiation, the cells were pulsedlabelled with \(^3\)H-thymidine. Immediately following irradiation, the cells were incubated with 1 or 2 mM caffeine in the presence or absence of the four deoxyribonucleosides (dT\(_5\); concentration 0.1 mM each). After different incubation times (3-24 hr), chromosome preparation was performed. The percentage of cells with generalized chromosome shattering (fragmentation and/or pulverization of all chromosomes of a mitotic cell, GCS) and of cells with micronuclei was determined. In the absence of dT\(_5\), a strong synergistic effect of uv-irradiation and caffeine was observed. The addition of dT\(_5\), however, significantly reduced the potentiating effect of uv plus caffeine. The evaluation of mitotic indices and of cell cycle parameters obtained from autoradiographs (percentage of labelled mitoses) indicates that the antagonist effect of dT\(_5\) was not due to enhanced interphase death. Concerning the temporal correlation between cells with GCS and micronucleated cells, the data clearly indicate that GCS precedes the appearance of micronuclei.

**Cf-2**

**UV DOSE DEPENDENT INDUCTION OF ELECTROPHORETICALLY DETECTABLE MUTANTS IN CHO CELLS.** M. J. Siciliano and R. M. Humphrey, The University of Texas System Cancer Center at Houston and Smithville, Tx. In a series of 23 experiments CHO cells were subjected to 4 different doses of UV irradiation accounting for survivals of 100% (unirradiated controls), 30-50%, 10-20% and ~1%. Following irradiation cells were allowed to divide twice and then were then cloned. 1304 single cell clones were grown up and each analyzed electrophoretically for variation at ~ 40 enzyme loci. A total of 53413 loci were screened. Clones containing variants were subcloned to establish the heritability of the variants. 26 inherited variants (mutants) were isolated from the UV exposed groups (37327 loci screened) and only one from unirradiated controls (out of 16086 loci screened --p<.003). The 27 mutants fell into 4 phenotypic classes: 1. electrophoretic shift (13), 2. isozyme loss (10), 3. isozyme reexpression (2) and 4. post-translational modification (2). Frequency of Class 1 mutants peaked at 10-20% survival (6.0 x 10\(^{-4}\)/locus screened) and was significantly higher (p<.02) then in the highest dosed group (0.7 x 10\(^{-4}\)) indicating the sensitivity of electrophoretic shift phenotypes in detecting mutations induced by lower mutagenic doses. The high frequency of the induction of these electrophoretically detectable mutations in somatic cells when compared with the low incidence of such phenotypes induced via germ line mutagenesis (Soares, 1979. Env. Mut. 1:19) suggests the greater clinical relevance of somatic cell mutagenesis. (Supported in part by ES 01287).
Cf-3
DNA DOUBLE STRAND BREAKS IN MAMMALIAN CELLS AS MEASURED BY NEUTRAL FILTER ELUTION. Matthews O. Bradley, Merck Institute for Therapeutic Research, West Point, PA 19486.

Little is known about DNA double strand break (DSB) production and repair in mammalian cells. Because of the potential importance of DSBs in cytotoxic, mutagenic, and carcinogenic phenomena, a filter elution method has been developed for measuring DNA DSBs. The DSB assay is based on the alkaline elution procedure. Mammalian cells are placed on a 2 μ polycarbonate filter, lysed with a solution containing 0.05 M Tris, 0.05 M glycine, 0.5 mg·ml⁻¹ proteinase K, 2% w/v sodium laryl sulfate, pH 9.6. This solution is pumped over the cells at 0.35 ml·hr⁻¹ for one hour. The DNA is eluted from the filters with the same solution minus the proteinase K for 15 hours. The results show that the assay can detect DSBs induced by as little as 1 krad of X-ray. The rate of DNA elution through the filters at pH 9.6 increases with X-ray dose. X-ray produces approximately one DSB to every 20 single strand breaks (SSBs), whereas bleomycin produces approximately one DSB to every 5 SSBs, and H₂O₂ about one DSB to every 2000 SSBs. The introduction of double strand cuts by HpAI restriction endonuclease in DNA lysed on filters results in a rapid rate of elution under neutral conditions, implying that the method can detect DSBs if they exist in the DNA. The eluted DNA bands with a double stranded DNA marker in cesium chloride. This evidence all suggests that the assay detects DNA DSBs. L1210 cells can rejoin DNA DSBs induced by 5 to 10 krad of X-ray with a half time of about 40 minutes. Given the apparent reproducibility and sensitivity of this assay, we expect to be able to examine a number of questions related to the biological consequences of DSBs in mammalian cells.

Cf-4
EFFECT OF X-RAY DOSE FRACTIONATION ON THE FREQUENCY OF RECESSIVE MUTATIONS INDUCED IN OOGONIA OF DROSOPHILA. H.U. Meyer and S. Abrahamson, University of Wisconsin, Madison, WI 53706.

We have recently accumulated an extensive body of data on the frequencies of sexlinked recessive lethals induced by x-rays in Drosophila oogonia. These data, for single-dose ("acute") exposures ranging from 20 to 6000 R, can best be accounted for by a linear-quadratic model which describes the mutational yield (Y) for a particular dose (D) as $Y = C + aD + bD^2$, where C (the control) is $1.29 \times 10^{-3}$, $a = 2.81 \times 10^{-6}$ and $b = 6.28 \times 10^{-10}$ as obtained from the data by weighted regression analysis. This implies that the yield of mutations for high total doses should be influenced by the mode of dose delivery; it should be greater for single acute exposures than when the dose is delivered in fractionated or protracted exposures. Such experiments were performed, using various patterns of fractionation encompassing seven days.

These are the data for sexlinked recessive lethal frequencies with standard errors (in %): 6000R, acute, 3.86 ± .15; 2 fractions of 3000R, 3.53 ± .11; 3 x 2000R, 2.83 ± .19; 4 x 1500R, 3.28 ± .18. 4500R, acute, 2.85 ± .12; 3 x 1500R, 2.38 ± .13; 9 x 500R, 1.85 ± .11. These data then support the validity of the proposed model equation, indicating that recessive mutations can result both from 1-track and 2-track ionizations, the latter becoming increasingly important at high doses.

Research support by ERDA & DOE (EY-76-S-02-2001), and by NIH (GM15422).
Cf-5
POSITION-SPECIFIC EFFECTS OF TRITIUM DECAY IN MATURE SPERM. W.R. Lee and P.M.S. Skinner, Louisiana State University, Baton Rouge, LA 70803
To determine if transmutation of tritium to helium-3 at specific positions in DNA has genetic effects not solely attributable to beta radiation the frequency per tritium decay of sex-linked recessive lethal (slrl) mutations and loss of sex chromatin has been measured in Drosophila melanogaster. Sperm with tritium in specific positions of DNA were obtained from males which, as larvae, had been fed tritium-labeled DNA precursors. Sperm transferred during mating were either used immediately for fertilization or stored in the seminal receptacles for 20-25 days. The increase in mutation frequency in stored vs non-stored samples from each treatment is due to \(^3\text{H}\) disintegrations which occurred during storage of the mature sperm. The following pattern of slrl frequency per tritium decay was observed (frequencies per decay are \(\times 10^5\)): 6-position of pyrimidines, 5.3; 8 of purines, 4.6; 5 of cytosine, 3.1; methyl of thymine, 1.4. As with prokaryotes there was an increase in mutation frequency per decay at the 5-position of dC over that for decay in the methyl group of dT. However, there was an even greater increase when the 6 (rather than 5) position of the pyrimidines was labeled. (8-\(^3\text{H}\))dC was the only precursor to give a significant increase during storage of loss of the X or Y chromosome and gave one of the highest frequencies of induced slrl's per tritium decay. Our results with (8-\(^3\text{H}\))dT are in contrast to those in prokaryotes where this compound gave no higher frequency of local effects than (me-\(^3\text{H}\))dT. Whether tritium is "more mutagenic" if incorporated at certain sites depends on the assay used to determine mutagenicity. Observed differences between systems may be due to differences in types of mutagenic lesions detected or to differences in the ability of the organisms to convert premutational lesions into mutations. (Supported by DE-AS05-76EV03728)

Cf-6
MUTAGENICITY OF HEAT AT THE AD-3 REGION OF NEUROSPORA CRESSA. M. D. Anderson, Illinois State University, Normal, IL 61761.
The mutagenicity of heat has been reported in bacteriophage, bacteria, and yeast. The phage T4 results implicate G-C base pairs as mutagenic targets for heat-induced transitions and transversions (Baltz et al., 1976, Proc. Nat. Acad. Sci. USA 73:1269; Bingham et al., 1976, Proc. Nat. Acad. Sci. USA 73:4159). The literature also suggests inhibited DNA synthesis, inhibited DNA repair, sister chromatid exchange, depurination, and chromosomal aberrations as other possible effects of heat on DNA. Drake and coworkers calculated high rates of mutation per diploid cell for higher organisms when they extrapolated the mutation rate per G-C base pair per day in phage T4 to larger genomes. They concluded that these high rates are biologically intolerable, and that a large percentage of the mutations are either prevented from occurring or are repaired. I have tested this hypothesis in the ad-3 forward-mutation system of Neurospora cressa. Resting conidia from a repair-sufficient strain (heterokaryon-12) and an excision repair-deficient strain (wus-2) (heterokaryon-59) were suspended in a pH 7, 10 mM sodium phosphate/MgCl\(_2\) buffer and exposed to 25, 35, 45, and 55°C in water baths. The heterokaryotic conidia and the total population of conidia were inactivated to the same degree at all temperatures tested, indicating that heat inactivates conidia by interacting with the cytoplasm rather than the nuclei. Heat was not found to be mutagenic at the ad-3 region in either strain. This result supports the hypothesis that higher organisms have evolved efficient avoidance and/or repair mechanisms that protect them from the mutagenicity of heat.
Ea-1

EFFECT OF EXTRACTS OF MUTAGENIC AND NONMUTAGENIC
FLY ASH ON MAMMALIAN CELL DNA

by
Facklam T. J., Crowley J. P. and Drum M. A.*

Aqueous extracts of a fly ash were found to be mutagenic in both microbial and mammalian cell systems. (Ames Salmonella sp. and Chinese Hamster V79 test systems.) Several other sample sets did not produce a positive response. These samples were evaluated as to their ability to interact with mammalian cell DNA through analysis of strand breakage by alkaline sucrose gradients, and the measurement of Sister Chromatid Exchange (SCE) frequency in Chinese Hamster Ovary (CHO) cells. Alkaline sucrose gradient analysis indicated that as the dosage increased, up to 600 µg/ml, there was a progressive shift of the DNA peak into the lighter region of the gradient indicating DNA strand breakage. For several nonmutagenic fly ash extracts no such shift in the DNA gradient profile was observed. Varying the length of time of incubation for the mutagenic fly ash extract also produced a gradual shift of the DNA into the light region over the course of 72 hours. The nonmutagenic fly ash extracts did not produce a attenuated DNA profile after 72 hours of incubation. A parallel indicator of chromosomal damage or interaction with an exogenous chemical was the measurement of SCE. CHO cells incubated for two hours with the nonmutagenic fly ash extracts at several dose levels, did not exhibit increased SCE frequency. The mutagenic fly ash was able to induce an increase in SCE frequency approximately twice that of controls. Our data suggest that a component of the mutagenic fly ash does interact with mammalian DNA.

Ea-2

CYTOTOXICITY AND MUTAGENICITY OF DIESEL EXHAUST SOOT EXTRACTS. A. P. Li, A. L. Brooks and R. E. Royer,* Lovelace Inhalation Toxicology Research Institute, P. O. Box 5890, Albuquerque, NM 87115.

The cytotoxic, mutagenic and chemical characteristics of extracts of diesel exhaust particles have been determined as part of a program to evaluate the potential health risks associated with use of diesel powered vehicles. The organic material from exhaust soot of a light duty diesel engine was extracted with two different solvents (methylene chloride and propanol-toluene) using two extraction procedures (ultrasonic and Soxhlet). Each of the resulting four crude extracts was separated into six different fractions through Sephadex LH-20 gel chromatography and the chemical composition was characterized. The cytotoxicity (using Chinese hamster ovary cells) and mutagenicity (Ames test) of each fraction were studied. Fraction 1 (aliphatic hydrocarbon) and Fraction 6 (hydrogen-bonding compounds) had low cytotoxicity and mutagenicity at the levels tested. Fraction 2 (aliphatic hydrocarbon) which also had a low mutagenicity, consistently had a higher cytotoxicity than Fraction 1. Fraction 3 (alkyl substituted aromatics) and Fraction 4 (polycyclic aromatic hydrocarbons, heterocyclics and quinones) were the most cytotoxic and mutagenic. Fraction 5 (polar compounds) had lower cytotoxic and mutagenic activities. Our data indicate that (1) the sephadex chromatography can fractionate the crude diesel extract into fractions with different biological activities, (2) there was a cytotoxic component (Fraction 2) which had no apparent mutagenic activity, (3) there seemed to be no major differences in biological activities of a given fraction when extraction was performed using the different solvents and techniques. (Research performed under U. S. Department of Energy Contract Number EY-76-C-04-1013).
Comparative Mutagenicities of MethylethylNitrosamine, Dimethylnitrosamine and Diethylnitrosamine. J. B. Guttenplan, NYU Dental Center, New York, NY 10010.

MethylethylNitrosamine (MEN) is a carcinogen which thus far has proved negative in liquid suspension and pour plate Salmonella typhimurium mutagenicity tests. Because of the similarity of MEN to dimethylnitrosamine (DMN) in structure, mutagenesis induced by MEN was carried out under conditions optimal for the detection of DMN induced mutagenesis. These conditions, pH 6.5, liquid incubation assay and the use of S-9 fraction from Aroclor induced mouse liver, resulted in appreciable mutagenesis in TA 1535 at 10 and 100 mM MEN. The time response for mutagenesis was biphasic with an apparent threshold time beyond which mutagenesis increased rapidly. This behavior is similar to that observed for DMN induced mutagenesis. DMN was about 1.5 x as potent a mutagen as MEN. Mutagenesis by MEN and DMN was less efficient at pH 7.4 than at 6.5. Only slight mutagenic activity was observed using diethylnitrosamine under these conditions. These results suggest that mutagenesis induced by MEN proceeds mainly via the same pathway as does mutagenesis by DMN, and that mutagenesis by N-nitroso methylalkylamines might best be detected under conditions that optimize mutagenesis by DMN. (Supported by NCI Grant No. CA 19023).

ADDITIONAL RESULTS FROM SPECIFIC-LOCUS TESTS ON THE SUPERMUTAGENICITY OF ETHYLNIITROSUREA IN THE MOUSE. W.L. Russell, P.R. Hunsicker and D.A. Carpenter, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

EthylNitrosurea (ENU) has been shown to be by far the most potent mutagen yet discovered in the mouse (Russell et al., 1979, Proc. Natl. Acad. Sci. 76:3818-3819). Additional results with this compound are presented here. Wild-type mice were injected intraperitoneally with ENU dissolved in phosphate buffer adjusted to pH 6. They were mated to a test strain carrying seven marker genes in homozygous condition, and the offspring were scored for mutations at the specific loci. With an injected dose of 250 mg/kg of ENU, 116 presumed mutations have now been observed in 20,501 offspring derived from cells exposed to the chemical in spermatogonial stage. The mutation rate is 19 times greater than that obtained with the most effective dose of procarbazine, heretofore the most potent mutagen known in the mouse. Preliminary data at 100 and at 50 mg/kg show approximately the same mutation frequency, on a per mg/kg basis, as that at 250 mg/kg. Offspring from cells exposed in post spermatogonial stages and from maturing oocytes in treated females both show a considerably lower mutation rate than that obtained for spermatogonia. This suggests that ENU may be most effective mutagenically in dividing cells, perhaps during DNA synthesis. [Research sponsored by the Dept. of Energy under contract with Union Carbide Corporation.]

CHEMICAL DOSIMETRY AND UNSCHEDULED DNA SYNTHESIS STUDIES OF ETHYLENE DIBROMIDE IN THE GERM CELLS OF MALE MICE. Gary A. Segal and Rene E. Sotomayor, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

(C3Hf×101)F1 males received i.p. injections of ethylene dibromide (EDB) ranging from 50 to 250 mg/kg. No clear-cut induction of unscheduled DNA synthesis (UDS) was observed in early spermatids at any of the tested doses although this germ cell stage has shown a strong UDS response to a number of chemical mutagens. Chemical dosimetry studies using [3H]EDB were also carried out. An i.p. injected dose of 5.2 mg/kg resulted in 46 alkylations/10^8 deoxynucleotides (dN) in testes DNA 4h after treatment compared
to $7\times 10^8$ dN in liver DNA. Label was gradually lost from liver DNA so that by 12 days posttreatment only 1/6 of the label found at 4h was still present. However, the labeling of testicular DNA did not appreciably decrease during this same time period, indicating that testicular DNA alkylation products were being removed very slowly, if at all. This result was in agreement with the negative UDS response. By sampling sperm moving through the epididymides and vas for 4 weeks after treatment it was found that mid to late spermatids were the most heavily alkylated stages although the alkylation of DNA in these stages did not increase.

The pattern of germ cell alkylation is reminiscent of that produced by ethyl methanesulfonate (Sega and Owens, Mutat. Res. 52 (1978) 87–106) and may reflect increased binding of EDB and metabolites to protamines in these stages. The dosimetry experiments showed alkylation of liver DNA to be -10x that for testes DNA which, in turn, was - 10x that found in the DNA of late spermatids and mature sperm. (Research sponsored by the Division of Biomedical and Environmental Research, U.S. DOE contract W-7405-eng-26 with Union Carbide Corporation and by DOE/EPA Interagency Agreement No. 78-D-X0453.)

Es-6

BENZENE METABOLITES INCREASE SISTER CHROMATID EXCHANGES AND DISTURB CELL DIVISION KINETICS IN HUMAN LYMPHOCYTES.

K. Morimoto and Sheldon Wolff, Laboratory of Radiobiology and Department of Anatomy, University of California San Francisco, San Francisco, CA. 94143

Benzene, which has been associated with human malignancies, is metabolized to produce several major metabolites that could be responsible for the biological effects. Tests have been carried out on human lymphocytes in culture to determine if benzene or its metabolites, phenol, catechol, and hydroquinone induce cytogenetic changes and affect cell division kinetics. Whole blood was incubated for 72 hr in medium containing 20 µM BrdUrd and each compound to be tested at various concentrations ranging from 1.6 µM to 25 mM. The results indicate that benzene itself does not induce SCEs or affect cell division kinetics over a wide range of doses. Phenol only has an effect at very high doses. On the other hand, catechol is a potent compound that induces SCEs and delays cell division very readily; for example, 0.2 mM catechol increased SCEs more than five times above the control level, and caused a 24-hr delay in cell cycle times. Hydroquinone was also shown to be potent but less so than catechol. These data lead to estimates of concentrations required to induce either a doubling in the number of SCEs, a 12-hr delay in cell cycle times, or a 50% decrease in mitotic indices. The analysis of these concentrations suggested that the metabolites of benzene, especially catechol and hydroquinone, could be responsible for cytogenetic and cytotoxic effects of benzene in the body.

Eb-5

NEUROBLASTS OF THE GRASSHOPPER EMBRYO AS A NEW MUTAGEN TEST SYSTEM.

Jan C. Liang and Mary Esther Gaulden, Radiation Biology, Department of Radiology, University of Texas Health Science Center at Dallas, Texas 75235.

Neuroblasts of the grasshopper embryo (Chortophaga viridifasciata) in vivo are extremely sensitive to radiation, 1 R of x-rays inducing a detectable number of acentric chromosome fragments (Gaulden and Read 1978 Mutat. Res. 49:55). Because they have no spontaneous breakage, neuroblasts permit easy detection of clastogenic agents. For testing chemical
mutagens, an in vitro method has been developed which does not alter embryonic growth over a period equal to 5 cell cycles (1 cycle=4 h at 37°C). Validity of the method has been examined by comparing dose-response of chromosome aberration frequency at low doses of x-rays in vivo and in vitro; no significant difference was found.

Mitotic inhibition and cell death may affect the frequency of chromosome aberrations observed at given times after exposure to an agent. The neuroblast system is advantageous in that effects of a chemical on cell cycle progression and cell death can be accurately and easily determined by observations on individual cells in hanging-drop preparations. Results will be reported on the dose-response of chromosome aberration frequency in the neuroblast after exposure to a known chemical clastogen as well as effects of mitotic rate and survival over several cell generations. The high radiosensitivity of neuroblasts, including those of mammals, suggests that they may also be very sensitive to chemical agents. The grasshopper neuroblast is a promising system for testing potential mutagens and teratogens.

EB-2
CLUSTERING OF SEX-LINKED RECESSIVE LEthal MUTATIONS IN MULLER-5 TESTS IN DROSOPHILA. Alan J. Katz, Illinois State University, Normal, IL 61761.

Clustering of lethal mutations within a half-sib family in Drosophila is generally thought to result from a spontaneous pre-meiotic mutation occurring in the germ line of the parental male. The mutations within such a cluster would not be derived from independent mutational events, and the mutations would generally be deleted from the results and excluded from further analysis, particularly if observed in post-meiotic broods. The objective of this project is to examine alternative explanations of the clustering phenomenon in an attempt to evaluate the rationale of deleting clustered mutations from one's results. The alternative explanations are similar in that they assume the mutations within a cluster are independent of each other and include (1) variation in exposure to the mutagen among the treated males, (2) variation in sensitivity to the mutagen among the treated males, and (3) chance deviations from an expected Poisson distribution. Explanations 1 and 2 were examined by means of computer simulation, and it was found that relatively minor variation in exposure and/or sensitivity to a mutagen among treated males could produce substantial clustering of mutations. The degree of clustering increased with increased mutagenic potency. Explanation 3 is based upon the observation that clustering of mutations in Muller-5 tests is relatively uncommon. For example, if significant clustering of mutations is observed in approximately 5% of all assays, and if one employs a 5% significance level, then it is likely that most - if not all - of the mutational clusters result from type-I errors. It is concluded that clustering of mutations in Muller-5 tests does not necessarily imply that the mutations are derived from the same mutational event. To reduce the risk of deleting valuable data, it is recommended that one employ reduced significance levels (no greater than 1%) when examining for clusters.

EB-3
AN ASSAY FOR THE DETECTION OF INDUCED ANEUPLOIDY AND CHROMOSOME BREAKAGE IN DROSOPHILA. P.A. Foureman, University of Wisconsin, Madison, WI 53706

The likely existence of chemicals that exclusively induce aneuploidy necessitates the development of efficient detection systems for this endpoint, which is often neglected in mutagen screening. To this end, a single generation assay in Drosophila is described. The male parent bears a translocation involving the X and Y chromosomes and the female parent bears normal free-X chromosomes. All zygotes receiving the hypoploid paternal sex chromosome and a normal euploid maternal complement will die as a result of genetic imbalance. All survivors can be classified
as having resulted from (1) nondisjunction in either parent, (2) gross
deletion of the paternal X;Y chromosome, or (3) loss of the paternal X;Y
chromosome. X-ray experiments have been successful in readily detecting
gross chromosomal deletion with exposures as low as 100R and chromosome
loss with exposures as low as 200R to adult males. Following 500R
exposure to larval males, significant increases in meiotic nondisjunction,
chromosome loss and gross deletion values were detected in the exposed
F₁ relative to the control. Maternal exposures of 1800R resulted in a
significant increase over the control in X nondisjunction. Significant
increases over the control values for chromosomal loss and deletion
have also been obtained following exposure of adult males to EMS. These
results indicate that the screening system described is an efficient assay
for gross deletional and nondisjunctional events. (This work was
supported by grant GM-07133 of the N.I.H.)

Eb-4
A MUTANT IN DROSOPHILA WHICH INCREASES THE FREQUENCY OF DISOMIC EGGS
James M. Mason, National Institute of Environmental Health Sciences,
Research Triangle Park, NC 27709 intr. by R. Sharma

A new mutant has been identified in Drosophila which, in the presence
of the meiotic mutant ord, increases the frequency of disomic eggs without
affecting the frequency of nullosomic eggs. This mutant (diplo-producer,
symbol: Dip) is dominant and maps to the proximal region of chromosome-2.
Dip has no apparent effect in the absence of a meiotic mutant. However, in
Dip ord/+ ord females the total nondisjunction frequency is increased to
about 60% where the theoretical maximum is 50%; and disomic eggs are twice
as frequent as nullosomic eggs. This effect is not due to directed segre-
gation at either the first or second meiotic division because females that
are homozygous for the centromeric region as well as females that are
heterozygous for the centromere are produced. The X and fourth (and
possibly all) chromosomes are affected in females. However, Dip has no
effect in males. These results suggest that Dip increases the number of
chromosomes in the oocyte nucleus either through mitotic nondisjunction or
extra chromosomal replication.

Eb-5
FURTHER DEVELOPMENT AND VALIDATION OF A BIOCHEMICAL PROPAGAE INDUCTION
ASSAY FOR CARCINOGENS. R.K. Elespuru and R.W. Pennington,* Biological
Carcinogenesis Program, Frederick Cancer Research Center, Frederick,
Maryland 21701.

A prophage induction assay based on the expression of a lacZ gene
located adjacent to the lambda leftward promoter has been recently de-
teveloped.1 DNA damage by radiation or chemicals results in the induc-
tion of β-galactosidase, measured 2 to 5 h later in a colorimetric assay.
Current efforts are directed toward optimizing conditions for detecting
chemical carcinogens. Bacteriological media, activating enzyme sources,
and handling procedures were found to be important variables in the de-
tection of polycyclic hydrocarbons, aromatic amines, and nitrosamines.
Rich media and large amounts of Syrian golden hamster liver S9 were
found to enhance the activation of DMN, DEN, NPy, AAF, N-OH-AAF, AF, and
2-AA while inhibiting that of polycyclics in a colorimetric spot test on
agar. Minimal media and moderate amounts of rat liver S9 proved optimal
for the detection of BP and DMB. The use of gridded 100 mm square petri
dishes allows efficient testing of 36 solutions per plate. A large set
of carcinogens and related noncancerogens were tested for their ability
to induce prophage. The colorimetric spot test has also been adapted for

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bioautography, in which the active areas of thin layer chromatograms can be identified.


The mutagenicity test system with E. Coli K-12 (343/113) developed by G. Mohn for the detection of a wide spectrum of genetic changes was used to detect the mutagenic activity induced by a group of Aliphatic nitrosamines. Metabolic activation was incorporated into the assay by the addition of liver homogenates obtained from either Sprague-Dawley rats or C3H mice induced with 0.1% phenobarbital, in drinking water. Both dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) were mutagenic upon metabolic activation with a preference to revert the missense mutation at the arginine locus. DEN was capable of inducing the forward mutation selected as an ability to utilize galactose. DEN was converted effectively into a mutagen in a time period of 30 minutes to 2 hours while longer incubation (2-8 hours) was required for DMN. Metabolic activation with the mouse and rat liver preparations did not result in quantitative differences. Aliphatic nitrosamines that gave conflicting results with Salmonella assay (Rao, et al. Mutation Res. 66 (1979) 1-7) will be examined in the E. Coli system and the results will be discussed.

Research jointly sponsored by the Environmental Protection Agency (IAG-D5-E681; Interagency Agreement 40-516-75) and the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.


The addition of an exogenous metabolic activation system in the form of liver microsomal enzymes to cultured mammalian cells often results in sharply reduced mitotic activity. Although this effect can be minimized by pulse treatment for only a few hrs., typical in vitro cytogenetic studies should provide for exposure over a complete cell cycle, i.e. 12 to 24 hrs.

Recently, procedures have been developed for successful short-term cultivation of primary hepatocytes with intact metabolic capabilities. We have conducted studies to examine the feasibility of co-cultivation of primary rat hepatocytes with cytogenetic target cells, i.e. CHO cells. Hepatocyte concentrations up to 5x10^5/ml were compatible with mitotic activity in CHO cells seeded at 1.5x10^5/ml. A co-cultivation period of 24 hrs. was used in all experiments. Co-cultivation did not induce a significant increase in either structural chromosome alterations or SCE. Cytoksin at levels of 1 to 25 µg/ml produced a dose-related increase in SCE in the presence of hepatocytes. In the absence of hepatocytes, levels of 400-600 µg/ml were required to give a similar response. With co-cultivation, significant chromosome breakage was produced at levels of 25 µg/ml and higher. No chromosome damage was observed in the absence of hepatocytes at 1000 µg/ml. Dimethylnitrosamine (DMNA) was also tested in CHO cells both with and without hepatocytes. A clear dose-related increase in SCE and chromosome damage was observed in a titration up to 1000 µg/ml in the presence of hepatocytes. No response was observed in the absence of hepatocytes.
These studies indicate that co-cultivation of mitotically quiescent and metabolically competent rat hepatocytes with target cells may be a feasible model for screening compounds for induction of chromosome damage in vitro.

**Eb-8**

DETECTION OF UNSCHEDULED DNA SYNTHESIS IN RAT LYMPHOCYTES TREATED IN VIVO WITH CYCLOPHOSPHAMIDE AND TRIETHYLENEMELAMINE. M. J. Skinner, B. DeCastro; and J. F. Eyre*, McNeil Laboratories, Ft. Washington, PA 19034

Detection of unscheduled DNA synthesis (UDS) was used as an indicator of primary DNA perturbation by cyclophosphamide (CY) or triethylenemelamine (TEM). Five rats in each group were treated via an intraperitoneal injection of either CY (25, 50, 75, 100 or 150 mg/kg) or TEM (0.05, 0.5 or 5.0 mg/kg). Control groups were given intraperitoneal injections of sterile water equal in volume to the high dosage groups. Peripheral blood from each rat was cultured in the presence of $^3$H-thymidine; radioautography was used as an indicator system to detect $^3$H-thymidine uptake by lymphocytes undergoing UDS. A minimum of three hundred cells were scored for each rat; cells with greater than background but less than 50 grains were considered indicative of UDS. A significant (p<0.05 by Chi-Square) increase in UDS was observed in animals treated with CY and TEM. Many mutagens have been shown, in other systems, to stimulate UDS; an *in vivo* assay such as the one we describe supplies *in situ* metabolic biotransformation which enhances the usefulness of the assay. The ease and rapidity with which our system can be done makes it a very practical and effective mutagenicity screen for detection of primary DNA damage.

**Eb-9**


The metabolism of aromatic amines in the liver is primarily detoxification through ring hydroxylation and conjugation. Intact cell systems preserve both detoxification and activation pathways (Cancer Letters 5:81, 1978) and thus are useful for the evaluation of tissue profiles of metabolism and capability for the production of mutagens. For this purpose, we have developed an HGPRT mutagenesis assay (Mutation Res. 58:339, 1978) using rat liver epithelial cells which possess intrinsic metabolic capability. In the present communication, the mutagenicity of several N-substituted aryl compounds was examined. NOH-AAF was the most active, while 2AAF was more active than 2AAF and 4AAF was nonmutagenic.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (M) (24 hr exposure)</th>
<th>TG' Mutants/10^6 CFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-aminofluorene (AF)</td>
<td>10⁻³</td>
<td>toxic</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>57 ± 24</td>
</tr>
<tr>
<td>2-acetylaminofluorene (AAF)</td>
<td>10⁻³</td>
<td>77 ± 12</td>
</tr>
<tr>
<td>N-OH-AAF</td>
<td>10⁻⁴</td>
<td>toxic</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>108 ± 38</td>
</tr>
<tr>
<td>4-AAF</td>
<td>10⁻³</td>
<td>4 ± 6</td>
</tr>
<tr>
<td>none</td>
<td>--</td>
<td>2 ± 5</td>
</tr>
</tbody>
</table>

Furthermore, the mutagenicity of N-OH-AAF was inhibited by paraoxon. Thus, the ARL/HGPRT assay is sensitive to activation-dependent carcinogens and by providing intact cellular metabolism would strengthen results obtained in tests dependent upon subcellular metabolism.
Eb-10
2-DEOXYGALACTOSE RESISTANCE IN CHINESE HAMSTER OVARY CELLS. M. J. Grant and J. D. Irr, E. I. du Pont de Nemours & Co., Wilmington, DE 19898
CHO cells deficient in galactokinase activity are resistant to 2-deoxygalactose (DGa). The increased frequencies of DGa resistant colonies after mutagen treatment are consistent with mutational events at a haploid or hemizygous locus. In initial experiments the standard CHO/MGPT mutagenesis protocol was followed except DGa was the selective agent instead of 6-thioguanine. Cells grown in T flasks containing HEPES buffered F12 medium with 10% dialyzed fetal calf serum were exposed to ethyl methanesulfonate (EMS) for 18 hours and maintained in exponential growth until selection on day 8. Mutation frequencies were determined one week later. A linear dose response was observed. Five hour exposures to benzo[a]pyrene and N-nitrosodimethylamine (DMN) were tested with the rat liver activation system in HEPES buffered serum-free medium. Both compounds were converted to mutagens in this assay. Expression time experiments were conducted with EMS treated cells, selecting intermittently on days 1 through 8. Mutagen induced resistant colonies began to appear by day 3 but their frequency decreased by day 8. Cells treated with 0 and 333 μg/ml EMS and 30 mM DGa on day 8 exhibited mutation frequencies of 2.7 and 85.5 x 10^-6 respectively, while day 4 selection frequencies were 3.4 and 118.0 x 10^-6. Relative plating efficiencies of a mutant cell population were not affected when cocultivated with up to 5.0 x 10^5 wild type cells. Consequently, selection can occur at 5.0 x 10^5 cells per plate. This assay has a short expression time and provides an additional locus for routine screening.

Eb-11
DEVELOPMENT OF PRACTICAL METHODOLOGY FOR THE USE OF THE C3H 10T1/2 CELL TRANSFORMATION ASSAY IN AN INDUSTRIAL LABORATORY. Kay L. McCarthy and H. E. Scribben, Toxicology Department, Rohm and Haas Company, Spring House, PA 19477
A series of experiments were performed to validate the C3H 10T1/2 Cell Transformation Assay in our laboratory, as part of a battery of short-term tests to assess the carcinogenic potential of chemicals. A number of compounds which included benzo(a)pyrene, benzo(e)pyrene, 2-aminoanthracene, 1-aminoanthracene, acridine orange, ethionine, mitomycin C and nickel sulfate were tested according to a standard protocol. Dose levels, which yielded from >90% to <50% survival, were selected for the cell transformation assay on the basis of a toxicity test. Cell transformation test plates were stained after 6 weeks and scored for transformed foci by the criteria of Reznikoff, et al., (Cancer Res. 33: 3238-49, 1973). The variation in results within an experiment was examined by treating 12 identical groups (20 plates each) with a nontoxic, transforming dose of 3-methylcholanthrene (1 μg/ml). 77.6 ± 8.3 (S.D.) percent of the plates contained type III foci (1.65 ± 0.32 (S.D.) type III foci per plate); whereas both untreated controls (n=34) and solvent controls (n=40) contained no type III foci. When tested by chi-square analysis the results of the 12 groups, which ranged from 70 to 95 percent of plates with type III foci, did not differ significantly from each other. At the frequency of transformation in this experiment, the binomial distribution would predict a 1 x 10^-13 probability of finding zero transformed foci in 20 plates. Other experiments examined the effects of lowering the serum concentration to 5% after 10 days incubation or staining the plates after 5 weeks instead of 6. These changes in protocol were not adopted because they reduced the amount of transformation observed.
MORPHOLOGICAL TRANSFORMATION OF C3H/10T1/2 CL8 CELLS BY ALKYLATING AGENTS.

The standard method for C3H/10T1/2 CL8 (10T1g) cell transformation can not adequately detect alkylating agents (Reznikoff, et al., Cancer Res. 33, 3239, 1973). However, Bertram and Heidelberger (Cancer Res. 34, 526, 1974) have described a modification of the standard procedure in which an alkylating agent gave a positive result. This technique can be useful to laboratories that routinely use 10T1g cell transformation to test novel chemicals for carcinogenic potential. We have evaluated the method in our laboratory. The assay is run in two phases, a test of cytotoxicity to choose the correct concentration range and the actual transformation assay. In the first phase, a range-finding toxicity test was performed using several concentrations of an alkylating agent on culture dishes that contained synchronized cells (10^4 to 10^5 cells/dish). The cell numbers and alkylating agent concentrations that kill approximately 99.0 to 99.9% of seeded cells were selected for the cell transformation assays. In the second phase, cell transformation assays were performed at one or two selected levels of cell numbers per dish and at three or four levels of alkylating agent concentrations. By using this method, NNNG at 2 μg/ml and at 10^5 cells/dish produced well-defined Type II and III transformed foci. These results were consistent with those obtained by Bertram and Heidelberger. β-Propiolactone at 30 μg/ml and at 5 X 10^4 cells per dish produced well-defined Type II and III transformed foci. Preliminary results from a transformation experiment with methyl nitroso-urea indicated the presence of transformed foci. These data and their implications for routine testing of novel compounds are discussed.


Employing benzo(a)pyrene as a promutagenic polycyclic aromatic hydrocarbon, we have studied conditions necessary to achieve near-maximum mutation induction in the CHO/HGPRT system by coupling with an S9 rat liver homogenate prepared from Aroclor 1254 induced male Sprague-Dawley rats. We investigated optimal concentrations and ratios of the S9 mix constituents for activation. Mutagenicity and cytotoxicity of benzo(a)-pyrene is not dependent on the calcium to phosphate ratios of the S9 mix, as has been demonstrated for dimethylnitrosamine. A plateau of maximal mutant frequency by benzo(a)pyrene is obtained after 2-3 h treatment; mutation induction reaches near-maximum at S9 protein concentrations of 0.12 - 0.18 mg of S9 protein per treatment (in 5 ml) and decreases at higher S9 protein level in contrast to dimethylnitrosamine mutagenesis. Determination of the mutagenicity of various hydrocarbons on the molar basis follows this order: benzo(a)pyrene > benzo(e)pyrene >> pyrene; 7,12-dimethylbenzanthracene > benzanthracene >> anthracene. Results from such studies suggest the need to consider the effects of ionic composition and S9 protein level in screening for the mutagenicity of promutagens and to be cautious in concluding the lack of mutagenicity of a test compound.

(Research sponsored jointly by the Environmental Protection Agency under Interagency Agreement DB-E681-A0 and the Office of Health and Environmental Research, U. S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation; JS83 is a Monsanto Postdoctoral Fellow.)
Ec-1

In our continued efforts to determine chemical structure-biological activity (mutagenic/carcinogenic) relationships, we have examined nitrosopyrrolidine and a group of its derivatives for mutagenic activity in the Salmonella histidine reversion assay. The nitrosamines exhibited a specificity to revert the missense strains (TA-1535 and TA-100) upon metabolic activation with phenobarbital induced rat liver homogenates (S-9). Nitrosopyrrolidine and 2,4 dichloronitrosopyrrolidine were mutagenic as well as carcinogenic. Substitution in the 2 and 5 positions with methyl groups has eliminated both carcinogenic and mutagenic activities. This observation is in agreement with our previous studies with the derivatives of nitrosopiperidine and nitrosopiperazine, that a block in the α-positions next to the N-nitroso group eliminated the biological activity. Substitution in the 2-position with a carboxyl group (nitrosoproline) and an addition of a hydroxyl group at the 4-position (4-hydroxynitrosoproline) has eliminated both mutagenic and carcinogenic properties. The carcinogenic nitroso-3-pyrrolone was not mutagenic while the non-carcinogenic N-aminopyrrolidine was mutagenic in the plate incorporation assay. The 3-hydroxynitrosopyrrolidine, a weak carcinogen was mutagenic only under conditions of liquid pre-incubation. These results not only indicate the usefulness of this assay as a prescreen for carcinogenic agents but also in understanding the chemical mechanisms involved in their biological activity. Research jointly sponsored by the Environmental Protection Agency (IAC-D5-E681; Interagency Agreement 40-516-75) and the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

Ec-2
MUTAGENICITY OF N-NITROSOPYRROLIDINE DERIVATIVES IN SACCHAROMYCES CEREVISIAE. F. W. Larimer, A. A. Hardigree, L. R. Dry*, and J. L. Epler, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. 37830.

N-nitrosopyrrolidine (NPY) and six of its derivatives were examined for mutagenic activity in Saccharomyces cerevisiae. Induced reversion of the his1-7 missense marker, and forward mutation to canavanine resistance (canF) were assayed. NPY and 3,4-dichloro NPY were directly mutagenic to yeast taken from exponential growth, and were also mutagenic to stationary phase yeast when exogenous metabolic activation was provided by phenobarbital-induced rat liver homogenate. 2,5-dimethyl NPY and 2-carboxy, 4-hydroxy NPY were not mutagenic. N-aminopyrrolidine, N-nitroso-3-pyrrolone and 2-carboxy NPY were weakly mutagenic in stationary phase yeast. The pyrrolone and amino pyrrolidine derivatives required metabolic activation, while 2-carboxy NPY was directly active. Research jointly sponsored by the Environmental Protection Agency (IAC-D5-E681; Interagency Agreement 40-516-75) and the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

Ec-3

Diethylstilboestrol (DES), a synthetic oestrogen, is known to induce cancer of the vagina in adolescent female offspring of treated mothers (Herbst et al., 1971, New England J. Med., 184:878-881). It has also been shown to induce aneuploidy in the bone marrow cells of mouse (Christman and Hinkle, 1974, Can. J. Genet. Cytol., 16:831-835). We have studied
mutagenicity of DES in the haploid yeast reversion assay using stationary phase and growing cells of strain XV185-14C of Saccharomyces cerevisiae. DES dissolved in dimethylsulfoxide induces reversions of the three markers, arg4-17, hisl-7, and hom3-10, tested in our study. In stationary phase cells DES weakly induces mutations only in the presence of the S9 microsomal fraction. When growing cells are treated without the S9 microsomal preparation, the three loci vary in their sensitivity to DES treatment. The markers arg4-17, hisl-7, and hom3-10 show up to 10-, 3-, and 2-fold increase in reversion, respectively, over the control mutation frequencies when incubated for up to 44 hours. Experiments have shown that the increased frequency of prototrophs was due to mutation induction rather than selection. We have also observed that the toxicity of DES is affected by the solvent used. When dissolved in 95% ethanol, DES is highly toxic to yeast cells, a concentration of 16 µg/ml killing 95-100% of cells in less than 4 hrs treatment. On the other hand, when dimethylsulfoxide is used as a solvent, a concentration as high as 2000 µg/ml is nontoxic even when incubated for 48 hours. (Research supported by a strategic grant from the Natural Sciences and Engineering Research Council and a contract from Health and Welfare Canada.)

Ec-4

MUTAGENICITY OF METAL SALTS IN THE L5178Y MOUSE LYMPHOMA ASSAY. I. J. Oberly, and C. E. Piper, Hazleton Laboratories America, Inc., Vienna, VA 22180.

Epidemiological and laboratory animal studies have provided evidence of the carcinogenicity of a variety of metals in our environment. However, aside from reports of the detection of primary DNA damage induced by metal compounds there is limited information available concerning their mutagenicity. In the present study, AlCl₃, CdSO₄, HgCl₂, K₂Cr₂O₇, MgCl₂, MnCl₂, NaAsO₂, Na₂HAsO₄, NaCl and Pb(NO₃)₂, were tested for their potential to induce forward mutations at the thymidine kinase locus using L5178Y mouse lymphoma cells. The results can be grouped into three categories: (1) strong positive responses at survivals of greater than 10% from CdSO₄, K₂Cr₂O₇, and MnCl₂; (2) weak positive responses yielding 2 to 3 fold increases in mutation frequency (MF) above the solvent control at greater than 10% survival from HgCl₂, NaAsO₂, Na₂HAsO₄, and Pb(NO₃)₂; and (3) negative responses from NaCl, MgCl₂ and AlCl₃. At test doses resulting in low survival levels some metal salts in the two positive categories yielded enhanced mutation frequencies. However, other agents maintained weak positive responses at survivals less than 10%, and no significant increases in MF were obtained with NaCl at about 3% survival or MgCl₂ at less than 1% survival. The Mouse Lymphoma Assay appears to be very sensitive for metal salts. Mutagenic activity was detected at doses of less than 10µg/ml for all positive compounds except Pb(NO₃)₂ and Na₂HAsO₄. A difficulty encountered in these studies was the consistent attainment of test doses within the narrow ranges needed to demonstrate mutagenicity of generally accepted survival levels.

Ec-5

BACTERIAL MICROSPESUSPENSION ASSAYS WITH BENZENE AND OTHER ORGANIC SOLVENTS. N. E. McCarroll, C. E. Piper, B. H. Keech⁎, Hazleton Laboratories, America Inc., 9200 Leesburg Turnpike, Vienna, VA 22180

Five organic solvents were examined in bacterial microsuspension assays designed to detect interaction with genetic material. Serial dilutions of benzene, acetone, xylene, toluene and hexane were evaluated for primary DNA damaging activity using E. coli WP2 (uvrA⁺ recA⁺) and WP100 (uvrA⁻ recA⁻) and B. subtilis H17 (rec⁺) and M45 (rec⁻). Preferential killing,
as indicated by increased toxicity for repair deficient versus competent cells, was observed for WP100 and M45 following treatment with benzene. At a concentration of 0.069M, 18.7% of the WP100 survived. By contrast, 93% of the repair competent WP2 were recovered. The survivors of H17/M45 were not enumerated; however, semi-quantitative observations of growth correlated with the E. coli strains and suggest the requirement of a recombinational mediated function for repair of benzene induced damage. This hypothesis was supported by the results of a S. typhimurium microsuspension fluctuation assay. Forty-eight replicates of multiple serial dilutions of benzene were assayed for mutagenic activity using S. typhimurium strains TA1535, 98 and 100. In the presence of an Aroclor 1254 induced microsomal cofactor mix, 27%, 35%, and 29% of TA100 wells contained revertants to histidine prototrophy following 72 hours incubation with 0.069M, 0.034M and 0.017M benzene respectively, while growth occurred in 8% of the control wells. No conclusive evidence of genotoxic activity was obtained with the remaining solvents tested in the three microsuspension assays.

**Ec-6**

**MUTAGEN TESTING OF URINE SAMPLES FROM SCHOOL CHILDREN IN AN ISOLATED HIGH LUNG CANCER MORTALITY AREA.**  
G. R. Warren and S. J. Rogers, Montana State University, Bozeman, MT 59717

In an effort to determine the relationship (if any) between air quality and lung cancer risk in Montana, urine samples were obtained from school children (third and fourth grades) of areas of high risk with different epidemiological pictures and one area of low risk. Air filter samples were taken at the same times and both were analyzed for presence of mutagens. Children from Butte, MT (high risk, all inhabitants) manifested higher levels of mutagen than either Anaconda (high risk - occupational only) or Bozeman (low risk). Of 126 urine samples in Butte 11 had significantly elevated mutagen levels on TA1538 with S9 (aurochlor induced). Neither the 57 Anaconda samples nor the 30 Bozeman samples were positive. More samples are being obtained from Bozeman. Filter analysis showed both direct and indirect acting frame-shift mutagens present in Butte particulate. Filter loads were not always proportional to mutagen content. Anaconda and Bozeman had little or no mutagen present in any filter particulate. The identity of urine and filter mutagen(s) has not been established, but the activity as frame-shifters in both cases and a correlation between total air filter levels and total urine levels in Butte samples taken in two different seasons is suggestive. These data are a preliminary indication of the probable utility of such population screening in risk analysis.

**Ec-7**

**REVERSE PHASE HPLC ANALYSIS OF MUTAGENIC ACTIVITY IN CIGARETTE SMOKERS' URINE.**  
R. M. Putzrath and E. Eisenstadt. Harvard School of Public Health, Boston, MA 02115.

Cigarette smokers' urine is known to be mutagenic for Salmonella typhimurium strain TA1538 in the presence of rat liver postmitochondrial supernatant. To elucidate the chemical nature of the mutagen(s), organic extracts of smokers' urine have been fractionated and assayed for mutagenicity using strain TA1538. Urine was passed through columns containing Amberlite XAD-2 resin. The adsorbed material was eluted with acetone and partitioned between water and methylene chloride. Approximately 80% of the mutagenic activity but only 10% of the dry weight present in the acetone eluate was extracted by methylene chloride. This extract was subjected to reverse phase high performance liquid chromatography on C18 bonded silica. Although most of the material eluted in more polar fractions, the mutagenic activity was recovered among several relatively
non-polar fractions. The distribution of activity is qualitatively
similar among the several urines analyzed and suggests that the mutagen-
city extracted from cigarette smokers' urine by XAD-2 resin is due to a
mixture of several active substances.

Ec-8
THE MUTAGENICITY OF FRACTIONATED ORGANICS EXTRACTED FROM AMBIENT AIR.
Alan Kolber, T. Hughes, T. Wolff, Research Triangle Institute, Research
Triangle Park, NC 27709. M. Waters, L. Claxton, J. Huisingh, Genetic
Toxicology Division, Health Effects Research Laboratory, U.S. EPA, Re-
search Triangle Park, NC 27709.

Ambient air particulate was collected from five geographical locations
within the U.S. during 1977 and 1978. The organics were extracted with
cyclohexane-methanol and partitioned into six chemical classes, using a
chemical fractionation scheme validated with a mixture of known com-
ounds. The fraction of the mass of total organic extract was calcu-
lated for each chemical class, as well as total recoveries after partici-
tioning. The composition of each chemical class was examined by GC/MS
and each fraction assayed for mutagenicity using a modification of the
Ames Salmonella bacterial bioassay. The air was sampled from locations
near Houston and Beaumont, TX; Lake Charles, LA; Elizabeth, NJ; and
Upland, CA, using a Battelle High Volume (Maxi) Sampler for periods of
one week or more. The particulate was collected onto electrostatic
precipitator screens designed to separate the material into three size
categories (<1.7 μ, 1.7–3.5 μ, and >3.5 μ dia.). Mutagenicity assays were
quantitative (pour-plate) when sample size allowed and qualitative (agar
diffusion well-test) when sample size was limited. Site-related trends
for mutagenicity were observed. The polynuclear aromatic hydrocarbon
fractions were generally the most mutagenic for each site, although the
mutagenicity of other fractions varied from site to site. Supported
by U.S. EPA Contract 68-02-2724.

Ec-9
THE MUTAGENICITY OF DIALKYLAMINOALKYL CHLORIDES IN A BATTERY OF THREE
SHORT-TERM ASSAYS. Christina Thompson, Sharon Rinzel, Gregory Probst and
Robert E. McMahon. Lilly Research Laboratories, Indianapolis, IN 46285.

Dialkylaminoalkyl chlorides are valuable chemical manufacturing
intermediates. In the concentration gradient bacterial mutagen assay
(Cancer Research 39: 682, 1979), dialkylaminoethyl chlorides were detected
in WP2 and WP2 uvrA− below 1 μg/ml concs. They were also detected but at
higher concs. in TA1535, TA100 and G46. They were negative in C3076,
TA1537, TA1538 or TA98. In conc.-response studies in WP2 uvrA−, the rela-
tive activity was dimethyl > diethyl > pyrrolidino > morpholino > piperidino.
The same relative order of mutagenicity was observed in the production
of trifluorothymidine resistant mutants in L5178Y+/- cells. Dialkylamino-
propyl chlorides were weakly mutagenic in two bacterial strains, TA1535
and TA100 and showed no activity in WP2 or WP2 uvrA−. The dialkylamino-
ethyl chlorides were also evaluated for their ability to produce unsched-
uled repair DNA synthesis in primary cultures of rat hepatocytes. All
were active in this sensitive assay. The battery of three tests each
designed to detect "initiating" events appears to be very efficient in
detecting even relatively weak alkylating agents of which the above class of
compounds is representative.

Ec-10
IN VITRO MUTAGENICITY AND TRANSFORMING ACTIVITY OF ELECTROSTATIC COPY
IMAGER. Thomas H. Connor, and Jonathan B. Ward, Jr., Dept. of Preventive
Medicine and Community Health, University of Texas Medical Branch, Galves-
ton, TX 77550
Electrostatic copiers create photoreproductions by imprinting a finely powdered carbon imager on paper. Human exposure to imager can occur by its transfer from copies to hands and possibly by inhalation of airborn powder. Extracts of printed paper and imager were evaluated for mutagenicity and imager was tested for transforming activity. Acetone extracts of paper samples were tested with Salmonella typhimurium strains 1537, 1538, TA98 and TA100 for mutagenicity. Plain white paper and white copies run through a Xerox model 4000 copier were negative while extracts of copied text and totally exposed (black) paper were positive in all 4 strains. Chloroform was also an effective solvent while methanol and physiological saline were not. Dry imager (DeSoto, Inc., Garland, TX) produced a dose responsive increase in his r revertant frequency in TA98 of 3 to 11 fold over control at doses between 100 - 1000 µg/plate. Liver homogenate (S-9) with or without NADP sharply reduced mutagenic activity. The imager was tested for transforming activity with Balb3T3 A31-1-13 mouse embryo fibroblasts. Transformation frequency at 100 µg/ml was 4.0×10^4 survivors and 2.0×10^5 survivors in controls. Frequencies at lower concentrations were not significantly elevated over control levels. Transformed foci formed at all dose levels were tested for growth in soft agar and those from 330 and 1000 µg/ml treatments plated more efficiently (8-57% P.E.) than did foci from controls or lower doses (2-17% P.E.) suggesting that treatment stimulated progression to a more highly transformed state. The detection of mutagenic and transforming activities in electrostatic photocopies and in the dry imager used in copy making indicates a need for further evaluation of potential human exposure to significant levels of mutagens resulting from copying.

Ec-11

ETHYLNITROSOUREA IS HIGHLY POTENT IN THE MAMMALIAN SPOT TEST, L.B. Russell and C.S. Montgomery, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

It has been reported that ethylnitrosourea (ENU) is a supermutagen in the specific-locus test in spermatogonia of the mouse (W.L. Russell et al., 1979, Proc. Natl. Acad. Sci. USA, 76:5818). Recent evidence (W.L. Russell, these proceedings) indicates it to be less mutagenic in post spermatogonial stages and oocytes. -- We have tested ENU in our in vivo somatic-mutation system ("mouse spot test") which involves exposure of embryos heterozygous for most of the same markers that are used in the specific-locus test. Following development, a colored clone (spot) in an otherwise black coat indicates expression of a recessive due to mutation in, or loss of, its wild-type allele. -- Embryos were exposed to ENU on day-10½ postconception (i.p. injection of the pregnant C57BL mother). To date, 263 animals from treated groups have been observed. ENU causes no excess prenatal death at 50, 75, or (probably) 100 mg/kg. Postnatal survival to weaning was near-normal at 50, reduced at 75, and (probably) totally inhibited at 100 mg/kg. All 3 doses produced dose-related morphological anomalies. -- The frequency of mice with a recessive spot (RS) was 21.6 and 17.9% at 50 and 75 mg/kg, respectively. This is by far the highest RS frequency ever observed in our laboratory, paralleling the supermutagenicity of ENU found in spermatogonia. If, as is thought, about 200 melanocyte precursor cells are at risk at time of treatment, the observed per-locus rate in the spot-test is roughly equivalent to the spermatogonial rate. [Research sponsored in part by EPA (IAG-D5-E681) and in part by the Div. of Biomed. & Env'tl. Res., USDOE, under contract W-7405-eng-26 with the Union Carbide Corp.]

Ec-12

EVALUATION OF THE MUTAGENICITY OF n-BGE AND t-BGE IN A BATTERY OF SHORT-TERM ASSAYS. T.H. Connor, T.G. Pullin, J. Meyne, A.F. Frost, and M.S. Legator, University of Texas Medical Branch, Galveston, TX 77550

The mutagenicity of n-butyl glycidyl ether (n-BGE), a widely used difluent for epoxy resins, and t-butyl glycidyl ether, (t-BGE), a possible
replacement for n-BGE, was evaluated using a battery of short-term assays. These included: (a) the Ames test without activation and with both pheno- barbital-and Aroclor-induced S-9; (b) DNA repair in human lymphocytes; (c) analysis of the urine of mice treated with each agent; (d) the micronu- cleus test; (e) metaphase analysis. The in vivo assays were carried out on the same groups of mice which received 100, 200, or 400 mg/kg/day. The agents were administered orally in corn oil and were given on five con- secutive days with the urine being collected after the fourth treatment. It was determined that both agents produced only base-pair type mutations and further analyses were carried out on Salmonella stain TA1535. While both n-BGE and t-BGE are direct acting mutagens in vitro, n-BGE is some- what more mutagenic than t-BGE and the addition of the different S-9 pre- parations had varying effects on the mutagenicity of both agents. Only the urine of the mice treated with t-BGE demonstrated any mutagenicity when evaluated with both TA1535 and TA98 and only when β-glucuronidase was added. T-BGE, when administered for five days, produced no significant effects in either the micronucleus test or the metaphase analysis. In the DNA repair assay using human lymphocytes, both agents produced dose re- lated effects as determined by both scintillation counting and autoradio- graphy. Although n-BGE was consistently more toxic in the various assays and was more mutagenic than t-BGE in vitro, the interpretation of the over- all results indicate that both are capable of genetic damage.

Supported In Part By Dow Chemical USA.

1. t-BGE only.

EC-13

The enzyme lactate dehydrogenase-X (LDH-X) is a form of lactate dehydro- genase comprised of the LDH-C subunit and found only in sperm and sperma- tids. The objectives of this study are to develop a methodology and to investigate the spontaneous or induced derepression of LDH-X in single mouse hepatocytes. A positive response model was first developed using rabbit anti-mouse (DBA/2J) anti LDH-B in conjunction with fluorescein iso- thiocyanate (FITC) conjugated goat anti-rabbit IgG. For the test system, antibodies to purified mouse LDH-X (DBA/2J) were raised in horses; the IgG fraction was isolated and absorbed first on Sepharose 4B and then on mouse hepatocytes to remove non-specific reactivity. FITC conjugated goat anti- horse IgG was similarly absorbed. Preliminary results from screening mouse hepatocytes indicate that the frequency for expression of LDH-X in hapato- cytes from normal DBA/2J mice is approximately 7.6 x 10^-7. The develop- ment of this system has potential for use in studying the normal and ab- normal mechanisms of derepression and differentiation in mammals. Studies are now in progress to further evaluate the frequency of LDH-X expression in livers from normal, hepatectomized, and mutagen-treated mice.

ED-1
TESTS FOR MUTAGENICITY OF LAKE BLOOMINGTON WATER IN SALMONELLA and MAIZE.
M. W. Heartlein*, D. M. DeMarini, H. E. Brockman, A. J. Katz, M. J. Plewa, and J. C. Means, Illinois State University, Normal, IL 61761; 1University of Illinois, Urbana, IL 61801; and 2University of Maryland, Solomons, MD 20688.

Using the Salmonella/microsome test, we have tested for the mutageni- city of concentrates of treated (drinking) water and untreated (raw lake) water from Lake Bloomington, IL. Twenty liters each of treated and un- treated water were collected monthly from May through October, 1979, and 20 liters of distilled water were used as a control. Each 20-liter sample was passed through an XAD-2 column, each column was eluted with 20 ml of acetone, and the eluates were evaporated to dryness under nitrogen and redissolved in DMSO to yield 3000-X concentrates. The concentrates were

108
assayed in strains TA98 and TA100 of *S. typhimurium* in the preincubation method (Nagao *et al.*, 1977, Mutat. Res. 42:335) with and without S-9 mix prepared from Aroclor 1254-induced rat liver. One-tenth ml of each 3000-X concentrate was tested in duplicate in two experiments. DMSO (0.1 ml) was used as a solvent control. Generally, concentrates of treated and untreated water caused a small, but significant, increase in reversion in both strains with S-9 mix. Without S-9 mix, the toxicity of the concentrates generally prevented scoring for reversion. The concentrate of treated water in May was strongly mutagenic in TA100 with S-9 mix, resulting in ~3,500 revertants/plate. Unconcentrated water samples were tested chronically for mutagenicity in the *uvr*-90 reverse mutation test in *Zea mays* inbred M14. The frequency of revertant pollen grains from plants watered with treated or untreated water was not different from plants watered with distilled water. Chemical differences in the water concentrates have been revealed by gas chromatography/mass spectrometry.

**Ed-2**

**DIFFERENT MUTATIONAL RESPONSE OF ZEA AND TRADESCENTIA TO ENVIRONMENTAL POLLUTION.** Wm. R. Lower, Environmental Trace Substances Research Center, University of Missouri, RR3, Columbus, Mo. 65201.

We are engaged in mutagenic studies of a variety of situations of environmental pollution, in situ, under real world conditions. We have observed, in 1978 and 1979, differential response of *Zea* and Tradescantia to the mutational milieu of petrochemical complexes, a non-ferrous smelter and a research nuclear reactor/cooling tower. The differential response is very high mutation frequencies of *Zea* and moderate to low to possibly suppressed mutation frequencies in Tradescantia. These differences have been observed in plantings in contaminated soil, where both soil and air pollution are factors, and in clean soil in pots where air pollution is the factor. The following is an example from a petrochemical complex:

<table>
<thead>
<tr>
<th>Location</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zea pollen Count x 10^6</td>
<td>1.13290</td>
<td>1.00790</td>
<td>1.68244</td>
</tr>
<tr>
<td>(Mut. Freq. ± SE) x 10^-5</td>
<td>19.95 ± 3.42</td>
<td>76.65 ± 7.10</td>
<td>19.61 ± 4.92</td>
</tr>
<tr>
<td>Tradescantia* (Mut Freq. ± SE) x 10^-3</td>
<td>1.55±.12 (155)</td>
<td>1.56±.30 (27)</td>
<td>1.48±.11 (174)</td>
</tr>
<tr>
<td>(n= of mut. events)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The historical spontaneous mutation frequency of *Zea* is 4.64 x 10^-5. Our spontaneous frequency for Tradescantia is 2.40 x 10^-3. The cause(s) of the differential response are not known, but it is conjectural that differential response to physiological toxicity may be one factor. Although, at the moment, no genetic mechanism is known to also contribute to the differences, the differences modify the comparative use of the two systems for *in situ* monitoring.

**Ed-3**

**FORWARD MUTATION IN INBRED EARLY-EARLY SYNTHETIC, ZEA mAYS BY CHRONIC EXPOSURE TO EMS.** M. J. Plewa, M. L. Ho*, P. A. Dowd*, and E. D. Wagner*, University of Illinois, Urbana, IL 61801.

We analyzed pollen grains for forward mutation at the *uv* locus in a rapidly maturing inbred of *Z. mays*. The purpose of the study was to develop an eukaryote, specific locus assay with a high degree of genetic resolution for chronic exposure to mutagens. Early-Early Synthetic matures from kernel to anthesis in approximately 4 weeks and attains a height of 0.5 m under optimal growing conditions. Tests were conducted by applying 50 ml solutions of ethylmethanesulfonate three times a week to the soil of individual plants grown in pots with a diameter of 8 cm. The EMS concentrations ranged from 1 μM - 10 mM. Appropriate safety practices were followed and the treated plants were grown in a sealed and locked
environmental chamber. Control plants were grown in a separate chamber. Growing conditions included a 16 h photoperiod, equal amounts of fertilizer and water and temperatures for day and night of 25°C and 20°C, respectively. Approximately \(5 \times 10^5\) pollen grains were analyzed per group. In one experiment the frequencies of mutant pollen grains for controls and the 1 \(\mu\)M, 10 \(\mu\)M and 50 \(\mu\)M EMS treatment groups were 0.80, 1.84, 6.54 and 2.10 \(\times 10^{-5}\). Plants exposed to solutions above 50 \(\mu\)M EMS produced only aborted pollen grains; this may be due to induced recessive lethal mutations. The data from several experiments indicate a dose dependent response to increased EMS concentration. This inbred may be a useful assay for the chronic exposure of mutagens. Supported in part by NEHS grant No. 5 RO1 ES01895-02.

Ed-4

**ZEA MAYS AS A MONITOR FOR IN SITU MUTAGENESIS.** V. K. Drobeny, University of Missouri, Env. Trace Subs. Res. Cntr., Route #3, Columbia, MO 65201.

Zea mays, strain W-22 has been used by our laboratory since 1976 as an indicator of environmental mutagenesis. A variety of in situ situations have been investigated, with emphasis on Amax lead smelter, Bixby Missouri, St. Joseph lead smelter, Herculaneum Missouri and petrochemical complexes in Wood River, Illinois and Beaumont, Texas. At the lead smelters, Zea was planted in the spring in the native soil at varying distances from each smelter stack. Tassels were harvested at anthesis and scored for mutagenesis. Results thus obtained are indicative of both soil and airborne pollutants. Data from Amax smelter presents the most conclusive picture with mutation frequencies very high adjacent to the smelter and falling off rapidly with increasing distance. Mutation frequencies at St. Joseph, a much older smelter, were elevated over background levels but showed no clear relationship to a distance gradient. At the petrochemical complexes Zea was planted in both clean potting soil, to test airborne pollutants exclusively, and in the native soil. Results obtained in both cases were uniformly high, with particular sites exhibiting values from six to sixteen times over the historical spontaneous mutation frequency.

Ed-5

**CHROMOSOME BREAKAGE INDUCED BY MALEIC HYDRAZIDE IN CULTURED HUMAN LYMPHOCYTES AND TRADESCANTIA POLLEN MOTHER CELLS.** Ifitikharruddin Ahmed and Teh-shiu Ma, Department of Biological Sciences and Institute for Environmental Management, Western Illinois University, Macomb, IL 61455

A comparative study was carried out to determine the effect of maleic hydrazide (MH) on chromosomes of human lymphocytes and Tradescantia pollen mother cells. Chromosome and chromatid aberrations as well as gaps in human lymphocytes and micronuclei (MCN) in tetrads of Tradescantia were scored to reveal the quantities of chromosome breakage. Human lymphocyte cultures were treated with 10 and 20 \(\mu\)M of MH solutions. A positive control (300 R of X-rays) and a negative control were maintained. The average of 25.3 aberrations and 16.3 gaps per 100 metaphase figures were induced by 10 \(\mu\)M of MH; and 12.0 aberrations and 18.7 gaps per 100 metaphase figures were induced by 20 \(\mu\)M of MH. The positive control yielded 39.3 aberrations and 17.3 gaps and negative control yielded 2.7 aberrations and 2.0 gaps per 100 metaphase figures. Tradescantia plant cuttings were treated with MH solutions for 24 hr and results indicated an increase in MCN frequencies from 8.4 to 14.8 MCN per 100 tetrads when treated with 10 and 20 \(\mu\)M of MH respectively. Positive control (45 R of X-rays) and negative control yielded 78.7 and 4.6 MCN per 100 tetrads respectively. Mutagenicity of MH, at 10 \(\mu\)M concentration, is about 3 - 5 times as efficient in human lymphocytes as compared with that in Tradescantia pollen.
mother cells. Results of this study indicated, for the first time, that
NR can also induce chromosome breakage in human lymphocytes.

Research supported by US EPA Grant #R806422010

Ed-6
IN SITU MONITORING OF AIR POLLUTANTS AND SCREENING OF CHEMICAL MUTAGENS
USING TRADESCANTIA MICRONUCLEUS BIOASSAY, Te-Hsiu Ma, Van A. Anderson* 
and Iftikharuddin Ahmed, Department of Biological Sciences and Institute
for Environmental Management, Western Illinois University, Macomb, IL
61455

It has been reported that the Tradescantia micronucleus bioassay is
highly efficient in testing mutagenicity of gaseous (DBE, EMS, HN3) and
liquid (DMS, NaN3, cyclohexylamine, maleic hydrazide) forms of mutagens
and X-irradiation. In the present study, this bioassay was used for in
situ monitoring of air pollutants at industrial complexes, public parking
garages, truck and bus stops, and for testing of some selected chemical
mutagens. Monitoring was conducted by exposing the flower buds of Trades-
cantia plant cuttings to the atmosphere of the polluted site for 1 - 6 hr.
Chemical mutagen test was carried out by allowing the plant cuttings to
absorb the mutagen solution or the gaseous mutagen for 6 - 24 hr. The
treated and control samples were fixed and the frequencies of micronuclei
in tetrads were scored to determine the mutagenicity. A total of 11 sites
was monitored during the summer of 1979. Six sites yielded positive
results and 5 sites yielded negative results under each of their weather
conditions. Results of the tests on ascorbic acid (0.03 - 0.125%), pro-
ponic acid (0.0053 - 0.1%), Zinc chloride (1 mM) and hydrazine acid gas
(136 - 272 ppm) were positive under each of their experimental conditions
and dose levels. Tests on ascorbic acid and propionic acid without
buffer gave negative results and, at the same time, caused abortive tetrad
and microspores.

Research supported by US EPA Grant #R806422010

Ed-7
A PROBE OF MUTAGENICITY WITH THE PLANT ARABIDOPSIS, G. P. Redei, Intr. by
A. Eisenstark, University of Missouri, Columbia, MO 65211.

Criteria of usefulness of general mutagen assays involve (i) the detection
of most types of mutants, irrespective of the nature and mechanism of
the mutations, (ii) high sensitivity coupled with a low level of noise, (iii)
fast and low cost of the procedures and applicable year around both in the
laboratory and in the natural environment. These requirements can be satis-
fied by an Arabidopsis assay. The experimental data indicate the possibili-
ty of detecting forward mutations at the embryo stage at an estimated number of
of over 14,000 loci and an additional ca. 3,000 loci may display mutants during
later stages of development. Presumably, each locus contains hundreds of sites,
the total number of potential targets within a genome is in the range of
several millions. Since the germline in the mature seeds is represented by
two diploid cells, the number of these potential targets must be multiplied
by a factor of 4 when seeds are exposed to mutagens. In case the mutagen
is applied during later stages of the development, this correction factor
becomes larger. Seed exposure to 0.3% EMS resulted in mutation frequen-
cies up to 0.47 of the genomes when classified at the stage of immature
embryos and up to 0.59 when seedling mutants were also considered. The
spontaneous rate of mutation at these loci has not been determined yet but
the observed frequencies are very low. - Arabidopsis may complete its life-
cycle, within 5-6 weeks; therefore the mutagen assays are relatively fast
compared to other higher eukaryotes. When the embryo test is employed,
there is no need to plant two generations because the mutants can be scored within the fruits developing from the seeds (or seedlings) treated. Several individuals can be raised to maturity in 5" pots. This species is winter hardy even in the most northern climate, thus in situ tests can be performed even during the winter.

Ed-8
DETERMINATION OF BIOCHEMICAL AND PHYSIOLOGIC EFFECTS OF ENVIRONMENTAL CONTAMINANTS USING GLYCINE MAX. D. L. Batema, intr. by Dr. Shabig Sandhu, Univ. of Missouri, Environmental Trace Substances Research Center, R93, Columbia, MO 65201.

Potential environmental contaminants were monitored at a lead smelter, a petrochemical complex, and at other urban industries using Glycine max (commercial strain, Cobb) as an indicator. The effects of these industries were measured as alterations in soybean biochemical and physiological functions, specifically changes in carbohydrate synthesis, nitrogen fixation, protein synthesis, and energy production. At the lead smelter, sites were chosen at various distances (0.3 km to 11.4 km) from the center of smelting activity. Sites closest to the smelter had decreased carbohydrate synthesis, nitrogen fixation and energy production; whereas, sites farther from the smelter had more normal plant function. In most cases there was a near-linear relationship with plant function and distance from the smelter. These same biochemical and physiologic tests were then applied to petrochemical complexes and other industries associated with large urban centers. The relationship of impaired plant function and environmental contaminant affect was not as definitive as the gradient analysis at the lead smelter. This may be due, in part, to a limited data base to date. Analyses at petrochemical complexes are continuing, with the addition of another biochemical test to determine changes in protein synthesis.

Ed-9
RADIATION-INDUCED MORPHOLOGICAL MUTATIONS IN HAWORTHIA MIRABILIS CALLUS TISSUES. K.N.PANDEY* AND P.S.KAHLON, Tennessee State University, Nashville, Tennessee 37203 P.S.Sabharwal* and J.Calkins*, University of Kentucky, Lexington, Kentucky 40506.

The objective of this study was to investigate the effects of γ-irradiation on the induction of morphological mutations in culture conditions. Callus was obtained from inflorescence axes on modified Murashige and Skoog's agar medium supplemented with 0.2 mg/l naphthaleneacetic acid (NAA) and 0.2 mg/l kinetin (6-furfuryl aminopurine). Callus was maintained on the same medium supplemented with 1.5 mg/l NAA and 1.5 mg/l Kinetin. Tissues were irradiated with 60COγ-rays at the dose rate of 208 rad/min. Dosages varied from 100 to 3000 rad. After 16 weeks the frequency of differentiated plantlets were analyzed for morphological mutations. The results showed that γ-irradiation was very effective in the induction of morphological mutations such as albina, xantha, straita and robusta. The most potent dose for the induction of mutation in callus tissue was 600 rad. The data will be presented to show that 600 rad produced as high as 6% albina and 16% xantha plus straita mutation frequencies. These tests suggest that the tissue culture can be a valuable tool to obtain desired mutants in vitro.
CYTOLOGICAL STUDIES ON METHYLENE CHLORIDE. H.N.B. COPALAN, G.D.E. NJAGI; AND P. HONGO-ALLEGRO,* UNIVERSITY OF NAIROBI, DEPARTMENT OF BOTANY, P.O. BOX 30197, KENYA, NAIROBI. Methylene Chloride is chiefly used as a paint remover, a blowing agent in foams and is intended for use as a propellant for aerosol sprays. The biological data available shows that it is moderately toxic on inhalation and ingestion. A tolerance level of 250 ppm in air is claimed. We have studied the cytogenetic effects using Vicia faba root meristems. Rooted seedlings were treated with 0, 10, 5 x 10^2, 10^3, 5 x 10^3, 10^4, 5 x 10^4, and 10^5 ppm methylene chloride solution. Root samples where obtained after 0, 15, 30, 45, 60, 90, 120, 150, 210 and 240 minutes treatment and processed for cytological observation. Methylene chloride inhibits mitosis and also induces premature chromosome condensation, plasmolysis and pycnotic nuclei formation in Vicia faba. The effects are dependent on the duration of treatment. Dose dependence could not be properly calculated due to the rapid volatility of the compound. Methylene chloride proved to be highly toxic to both Drosophila and Salmonella. Hence mutagenicity of the compound could not be ascertained. Still, because of its acute cytotoxic effects on Vicia faba, methylene chloride warrants more study before widespread use.

QUANTITATION OF MUTAGENESIS IN PLANT CELL CULTURES, G. Weber and K. G. Lark, Intr. by Paul Hynds, Department of Biology, University of Utah, Salt Lake City, UT 84112.

We have measured frequencies of mutants induced by 5 mutagens (Ethyl methanesulfonate (EMS), Methyl methanesulfonate (MMS), N-Methyl-N'-nitro-N-nitrosoguanidine (NNG), hycanthone (1-[[2-(diethylamino) ethyl] amino]-4-(hydroxymethyl)-9H-thioxanthene-9-one, ultraviolet light) in cell suspensions of soybean. Whereas the spontaneous frequency was 1.2x10^-7, induced mutation frequencies ranged from a low of 3.6x10^-5 for EMS, a point mutagen, to a high of 10^-3 for Hycanthone, a frameshift mutagen. Using a newly developed plating system, we have measured the survival and the frequencies of mutations after different doses of mutagen.

The mutation selected displays a phenotype of rapid growth on maltose. Doubling time in suspension cultures is decreased from 100 hr to ca. 30 hr by the mutation. Both wild type and mutant cells grow on sucrose with a 24 hr doubling time. Thus lethality can be rapidly estimated by growth on sucrose whereas mutants are scored on maltose medium. The high frequency of mutations induced by Hycanthone suggests that the mutation is recessive. Moreover, U.V. induces mutations in haploid cells at doses which fail to induce mutations in diploid cells.


Mutagenesis Section, Environmental and Occupational Toxicology Division, Department of National Health and Welfare, Ottawa, Canada, K1A 0L2.

Previous studies (Nestmann et al, Cancer Res. 39:4412-4417 (1979)) showed that the mutagenicity of the dye Rhodamine B (90%, Sigma) in Salmonella with S9 was due mostly to impurities and that in vitro metabolic activation was required for this activity. This dye was also effective, with S9, in causing DNA damage detectable by alkaline sucrose gradient (ASC) analysis and in reducing survival in CHO cells. Further studies were undertaken to determine: 1) the effect of this dye on chromosome aberrations and sister chromatid exchanges (SCE) in CHO cells; 2) the influence of impurities in mammalian cells and; 3) the requirement

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for S9 in CHO cells. Rhodamine B (Sigma) caused a dose-related increase in the percentage of cells with chromosome aberrations (30% at 0.95 mM). While there was a dose-related increase in SCE, the maximum was barely double the control. Unlike in Salmonella, Rhodamine B (Sigma) was also active in CHO cells without S9. Its potency is decreased with S9 in terms of survival and chromosome aberration and is increased with S9 for DNA damage. Another Rhodamine B (laser grade, Eastman) showed no mutagenicity in Salmonella, presumably because it lacks the mutagenic impurities. However, this dye behaved almost exactly like the Rhodamine B (Sigma) in terms of cell survival, DNA damage, chromosome aberrations and SCE in CHO cells. Thus, mutagenic activity in CHO cells is unlike that in Salmonella because purity is not a factor and S9 is not required. Whether two different activities are involved or CHO cells metabolize the dye differently remains to be established.

**Fe-2**

**MUTATION FREQUENCIES OF SOME STANDARD GENETIC TOXICANTS OBTAINED WITH THE CHO/HGPRT SYSTEM EMPLOYING LOW-SERUM MEDIA AND VARIOUS CONDITIONS FOR EXOGENOUS (S-9) METABOLISM.** A.R. Malcolm, K.O. Cooper, and A.P. Agins*, Environmental Research Laboratory, United States Environmental Protection Agency, Narragansett, RI 02882.

CHO-K1 cells, adapted for growth in low-serum medium for isolation of auxotrophic mutants, were employed in simple, validating studies of the CHO/HGPRT System, using standard compounds. Ham's F12 medium (or variations) supplemented with 2 percent, commercially-prepared, dialyzed fetal calf serum, was used. Exogenous metabolism was provided with rat liver (S-9) homogenates obtained from a commercial supplier and prepared from animals exposed to Arochlor 1254 or other inducers. Ethylmethanesulfonate, 5-bromodeoxyuridine, mitomycin-C, N-methyl-N'-nitro-N-nitrosoguanidine, and nitrosomethylurea were significantly mutagenic and showed well-defined dose responses when tested as direct-acting compounds with 16-hour exposures. Using 5-hour exposures, mutagenic profiles of several compounds requiring metabolism (dimethylnitrosamine, benzo[a]pyrene, 2-nitrofluorene, 3-methylcholanthrene, others) were determined as functions of (1) various doses of compound with 0.2 mg (S-9) protein per ml of reaction mixture, (2) single doses of chemical with varying amounts of (S-9) protein, and (3) the pathway utilized to generate NADPH (glucose-6-phosphate plus cofactors or isocitric acid). Preliminary results suggest optimal (yielding maximum mutation frequency) amounts of (S-9) protein to be dose-dependent and perhaps compound-dependent. Mutation frequency also appeared to depend upon the NADPH-generating pathway, with glucose-6-phosphate plus cofactors generally yielding values several times higher than isocitric acid when (S-9) protein was varied between 0.2 and 1.2 mg per ml of reaction mixture. Data are presented and problems encountered discussed.

**Fe-3**

**ENHANCEMENT BY GLUTATHIONE OF THE MUTAGENICITY OF SELENIUM COMPOUNDS IN MAMMALIAN CELLS, R. F. Whiting, L. Wei and H. F. Stich, B. C. Cancer Research Centre, Vancouver, Canada V5Z 1L3**

The mechanism of selenium mutagenicity was examined in relation to its biological pathways of metabolism. Glutathione strongly enhanced the induction of unscheduled DNA synthesis (UDS) in cultured human cells by inorganic selenium compounds. In the presence of $10^{-3}$ M glutathione, high levels of UDS (74-114 grains per nucleus) were observed in cells treated with selenate ($10^{-3}$ M), selenite ($10^{-5} \times 10^{-4}$ M), and selenide ($10^{-5} \times 10^{-3}$ M). Glutathione at $10^{-3}$ M also enhanced the clastogenic and cytotoxic effects of selenite and selenate in Chinese hamster ovary (CHO) cells. Glutathione at $10^{-4}$ M or $10^{-2}$ M caused less enhancement of DNA damage and toxicity in both the UDS and chromosome aberration assays. In the absence
of glutathione, these inorganic selenium compounds induced low levels of UDS (about 10 grains per nucleus) and moderate frequencies of chromosome aberrations (up to 11%). Three organic selenium compounds (selenocystine, selenocystamine and selenomethionine) were also examined for the induction of UDS. No unscheduled DNA synthesis was detected in cells treated with selenocystamine or selenomethionine, with or without added glutathione. However, selenocystine alone at 10^{-4} - 10^{-3} M induced a low level of UDS, and glutathione enhanced the DNA-damaging effect of selenocystine. The maximum amount of UDS (22 grains/nucleus) occurred in the presence of 10^{-2} M glutathione. This was about 20% of that detected in cells treated with selenite and 10-fold lower concentrations of glutathione (10^{-3} M). The results suggest that reduction is involved in the conversion of selenium compounds to mutagenic forms. The active mutagens may be selenols, GS-Se^{-} from inorganic selenium and R-Se^{-} from organic selenium compounds. (Supported by the N.C.I. of Canada.)

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MUTAGENICITY AND ENHANCEMENT OF MUTAGENESIS BY 2,3,7,8-TETRACHLORODIBENZOFURAN. R. Schoeny, Dept. of Environmental Health, University of Cincinnati Medical School, Ohio 45267.

Polychlorinated biphenyls (PCB) are ubiquitous environmental contaminants. The commercially prepared PCB mixtures are contaminated with chlorinated dibenzofurans, including the highly toxic 2,3,7,8-tetrachloro-isomer (TCDF). As part of a study of biological effects of PCB components, TCDF was assayed for mutagenicity in the Salmonella/microsomal reversion assay. TCDF delivered in a benzene:isooctane (1:9) vehicle was non-mutagenic in direct assays using strains TA1535, TA1537, TA98, TA100 and TA1978. TCDF was also inactive when tested in the presence of S-9's from both uninduced rats and from rats treated with a variety of inducing agents, including TCDF. Preliminary direct Bacillus subtilis rec assays have been negative for both TCDF and octachlorodibenzo-furan. While TCDF is itself non-mutagenic, it can be a promoter of mutagen activation when used as a hepatic enzyme inducer. When 5 µg/plate benzo(a)pyrene was incubated with varying doses of S-9 from TCDF rats, activation, as measured by numbers of mutant colonies, was seen to be S-9 dose dependent. The initial rates calculated from the linear portion of dose response curves using variously induced S-9's gave the following comparative values: PCB (500 mg/kg), 4.64 revertants/µg microsomal protein; 3-methylcholanthrene (40 mg/kg), 11.59 revertants; TCDF (90 µg/kg) 5.16 revertants. There was no linear relationship for uninduced S-9. Lower inducing doses of TCDF produced intermediate responses. Effects of S-9 enzyme induction by TCDF have been observed using 2-aminoanthracene and 3-methylcholanthrene as pre-mutagens.

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Kidney microsomal enzymes from mature and immature Babl/c mice were used to investigate the possible role of testosterone on the biotransformation of DMN. Testosterone-treated and control male and female mice used in mutagenic and androgenic receptor studies. These studies indicated that control mature male kidneys showed a significantly greater potential to biotransform DMN than the kidneys of both mature females and the immature animals to activate DMN. Nuclear androgen receptors were detectable in control mature males only. Testosterone treatment resulted in significant accumulations of nuclear androgen receptors in the kidneys of both mature females and the immature animals. The levels of
n-demethylase was higher in the kidneys of mature males than mature females. No data is available on the level of n-demethylase after testosterone treatment. Therefore these data suggest an association among the level of testosterone in the kidney, the level of n-demethylase in the kidney and the potential of the kidney to biotransform DMN to its mutagenic intermediate by Babl/c mice.

P-1
SENSITIVITY OF DROSOPHILA MELANOGASTER TO LOW CONCENTRATIONS OF GASEOUS MUTAGENS: III. DOSE-RATE EFFECTS* P. G. Kale and J. W. Baum, Brookhaven National Laboratory, Upton, NY  11973.

Sex-linked recessive lethal mutations were induced in D. melanogaster males by chronic and acute treatments of gaseous 1,2-dibromoethane ranging from 2.3 to 31 ppm·hr. Acute treatments for each dose were made by increasing chemical concentration approximately 30 times with a concommitant decrease in exposure period. Germ cell stages, in order of decreasing sensitivity, were: spermatocytes, spermatids, and spermatozoa. The dose response relation was linear for all three cell types both for chronic and acute exposures. The difference in mutation induction by chronic and acute exposures was statistically insignificant except at the highest dose where acute treatment induced a greater number of mutations than chronic. Greater (acute vs. chronic) mutation induction in sperma tozoa, a metabolically inactive state, and the insignificant differences between the two types of treatments at lower doses leads to the conclusion that the dose-rate effect at high doses is due to systemic factors such as metabolic deactivation or elimination rather than repair of premutational damage in the target cells. The significance of these observations in risk assessment for environmental pollutants is discussed.

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P-2
NEUTRON-INDUCED SPECIFIC LOCUS AND X-LINKED LEthal MUTATIONS IN DROSOPHILA S. Abrahamson, C. DeJongh and S. Marino*

D. melanogaster oogonia have been irradiated with neutrons of the following energies and with several doses in the ranges indicated: .43 MeV (500-1900 Rad); .66 MeV (250-1500 Rad); 2 MeV (250-2500 Rad); 6 MeV (250-3000 Rad); and 14 MeV (250-3000 Rad). X-linked recessive lethals and specific locus mutations (8 recessive loci on the X-chromosome) were scored.

The two main purposes of these experiments have been (1) to determine RBE (with respect to X-rays) for neutrons of different energies indicated above; and (2) to determine whether the dose-frequency response curve for any neutron energy best fits a linear (Y=C+αX) or a linear-quadratic (Y=C+αX+αX²) equation.

RBE's were computed for the neutrons of various energies using the formula αN/αX, where αX is derived from a weighted linear-quadratic regression equation of X-ray data collected in a manner similar to the neutron data, and αN is derived from a weighted linear regression equation for each energy of neutron. The RBE's are as follows: .43 Mev-4.6; .66 MeV-4.0; 2 MeV-3.1; 6 MeV-2.9; and 15 MeV-2.0.

The observed lethal frequencies of each energy of neutrons were compared to projected linear and linear-quadratic equations. In several cases, the data suggest that a D² component is significant. However, in 2 MeV neutron data (the energy chosen to determine if a D² component
exists), the observed doses ±95% confidence limits overlap both projected linear and linear-quadratic values.

Specific locus mutation rates induced by neutrons in oogonia indicate the highest RBE for 0.68 MeV neutrons (no data 0.43 MeV) followed by 2 and 6 MeV respectively.

P-3
GAMMA-RAY INDUCTION OF HPRT DEFICIENT MUTANTS OF HUMAN LYMPHOBLASTOID CELLS
Harvey F. Thomas, Richard Rudersdorf*, and Robert DeMars, The Laboratory of Genetics, The University of Wisconsin, Madison, Wis. 53706

We wish to induce deletion mutations in human cells, which can then be used for diverse experiments, e.g., as non-reverting recipients of DNA in gene transfer experiments or as cells that express alleles to only one major histocompatibility complex. Basic work towards this objective was done by irradiation of human lymphoblastoid cell lines (LCLs) established from normal lymphocytes of unrelated individuals. LCLs were treated with doses of 200–600 rads at concentrations of 4x10^3 cells per ml. with a Mark I Model 30 cesium source with a delivery rate of 26,115 rads per hr. The irradiated and control cultures were maintained for periods of up to 21 days post irradiation at concentrations of 4–6x10^5 cells per ml. Cells were cloned in agarose growth medium over confluent 6-thioguanine resistant (6-TG^2) human fibroblasts previously irradiated with 4000 rads. Cells not under selection were cloned at 300 cells per dish and cells under 6-TG selection were cloned at 5x10^5 cells per dish. All dishes were fed on days 1, 6, and 12 after the initial plating. Relatively small differences in slopes and shoulders of survival curves sometimes resulted in large, e.g., 50 fold, differences in survival after large doses. Recovery of maximum cloning efficiency of irradiated cultures required 9–12 days in the three lines tested. In the two lines used thus far, gamma-ray induced frequencies to 6-TG resistance reached 1.1x10^-4 and 6.4x10^-5 per survivor after treatment with 400 rads when the surviving fraction was 0.9% and 0.25% respectively. Peak frequencies in mutants were reached at day 15 post irradiation. The presence of deletions in the mutants is presently being investigated.

P-4
MUTAGENICITY TESTING USING THE L5178Y/TK^+/− MOUSE LYMPHOMA FORWARD MUTATION ASSAY; COMPARISON OF ACTIVITIES OF METABOLIC ACTIVATION SYSTEMS.
D. T. Tajiri and A. D. Mitchell, Biochemical Genetics Department, SRI International, Menlo Park, CA 94025

To develop further and validate the L5178Y TK^+/− mouse lymphoma forward mutation assay, comparative mutagenicity tests were made with 16 chemicals: 2-acetylaminofluorene (2-AAF), benzo(a)pyrene (BAP), diethylnitrosamine (DEN), 7,12-dimethylbenz(a)anthracene (7,12-DMA), dimethylnitrosamine (DMN), diphenylnitrosamine (DPN), ethylmethanesulfonate (EMS), hycanthone, 3-methylcholanthrene (3-MCA), 2-methyl-4-dimethylaminoazobenzene (DAAB), methyl methanesulfonate (MMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2-naphthylamine, pararosaniline, phenanthrene, and pyrene. These chemicals were tested in the absence of metabolic activation (OMA), and in the presence of noninduced (NMA) and Aroclor 1254–induced (IMA) Fischer 344 male rat liver S-9 systems using NADP and sodium isocitrate as cofactors. Four compounds (EMS, MMS, MNNG, and hycanthone) were found to be ultimate mutagens, eliciting a strong mutagenic dose response under all three conditions, OMA, NMA, and IMA. Eight compounds (2-AAF, BAP, DEN, 7,12-DMA, DMN, DPN, DAAB, and 3-MCA) were promutagens, eliciting a strong mutagenic dose response only with NMA or IMA. The four remaining compounds (2-naphthylamine, pararosaniline, phenanthrene, and pyrene) were weak mutagens or promutagens, eliciting a weak mutagenic

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response. A study of reproducibility between experiments and variations in activity among different batches of liver S-9 preparations is discussed. (Supported by NCI Contract N01/CP-65854.)

P-5
CYTOGENETIC ANALYSIS OF THE L5178Y MOUSE LYMPHOMA MUTAGENESIS ASSAY SYSTEM. J. Hozier*, M. Moore-Brown*, B. Howard*, T. Danzyl* and D. Clive*. *Florida Institute of Technology, Melbourne, Fla., bEnvironmental Protection Agency, RTP, NC, cUniv. of Minnesota, Minneapolis, MN and dWellcome Research Laboratories, RTP, NC. The L5178Y mouse lymphoma mutagen assay quantitates the induction of thymidine kinase-deficient (TK−) mutants from a standard TK+ cell line. Recent research has been geared toward analyzing the hypothesis that this assay detects both point mutants (represented morphologically as "large" trifluorothymidine-resistant (TFT) colonies — λ) and more grossly damaged chromosomal mutants (represented as "small" TFT colonies γ-7°). We have determined the banded karyotypes of the parent TK+/− heterozygous cell line as well as its TK− mutants in order to relate the genetic and morphological properties of mutant colonies. The parental cell line displays karyotype homogeneity, all cells containing normal mouse chromosomes, readily identifiable chromosome rearrangements and cell line-specific marker chromosomes. The λ mutants appear karyotypically identical within and between clones and most importantly identical to the parental TK+− line. This is in marked contrast with γ mutants which show gross chromosomal changes. In several cases these abnormalities are seen to be associated with chromosome 11 (the location of the TK gene). The cytogenetic analysis remains compatible with the hypothesis that the inheritable differences in λ mutants and γ mutants are related to the type of genetic damage sustained, with λ mutants receiving minimal damage, possibly in the form of point mutations at the TK locus, while γ mutants receive damage to other genetic functions coordinately with loss of TK activity, resulting from grosser insult to chromosomal material.

P-6

Treatment with the oxidized form of chromium, Cr(VI) as K2CrO4, produced a concentration-dependent increase in mutation induction at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovary (CHO) cells as determined by the frequency of 6-thioguanine resistant (TG2) mutants. Cr(VI) showed a concentration-dependent increase in mutagenicity over a range of 0.25-2.0 μg/ml. Typical results of a 5 hour treatment with Cr(VI) induced a maximum of 75 TG2 mutants/106 clonable cells at approximately 20% cloning efficiency; the spontaneous mutation frequency is generally 0-10 mutants/106 cells. We observed a decline, rather than an increase, of mutation frequency when treatment at Cr(VI) concentrations caused a reduction of cellular reproductive capacity greater than 80-90%. However, the reduced form of chromium, Cr(III) as Cr(CH3COO)3, did not exhibit notable mutagenicity or cytotoxicity at concentrations to 20 μg/ml. While we presume the ultimate cellular form of chromium is the trivalent species, mutation induction by chromium seems to be sensitively modified by the initial oxidation state and by the cellular growth conditions during treatment. At Cr(VI) concentrations which inhibit cell growth and presumably DNA synthesis, mutation induction is lowered or not demonstrable. This may be consistent with the notion that metal mediated enhancement of DNA replicative errors accounts for one
mechanism of metal mutagenesis.
(Research supported jointly by EPA under Interagency Agreement D8-E681-A0 and by DOE under contract W-7405-eng-26 with the Union Carbide Corp.)

P-7

QUANTITATION OF MUTATIONS INDUCED BY METABOLICALLY ACTIVATED MUTAGENS IN NORMAL HUMAN FIBROBLASTS. D. J. Chen, R. T. Okinaka, and B. J. Barnhart, Genetics Group, University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545.

Most normal human fibroblasts in culture do not retain the ability to metabolically activate procarcinogens/promutagens. Therefore, only direct-acting mutagens have been successfully detected in human cell assays. A human fibroblast mutation assay incorporating rat liver microsomes is being developed. Three model procarcinogens [(B(a)P, 3MC, and DMN] have been utilized for initial studies. Cells are treated for 2 h with Aroclor-induced rat liver S9 preparations in the presence of NADPH regenerating system in serum-free MEM medium buffered with 20 mM HEPES at pH 7.6. At a constant amount of S9 protein concentration, we obtain a linear increase in mutagenicity as a function of promutagen dose. 3MC and B(a)P concentrations ranging from 0-5 μg/ml are able to increase mutations 10- and 18-fold, respectively. DMN concentrations ranging from 0-5 mg/ml increase mutation frequencies about 6-fold. The cytotoxicity of these chemicals is expressed as a linear dosage response under the same conditions. However, at high S9 concentrations a detoxification phenomenon occurs with B(a)P. Our results show that a rat liver microsomal activation system can be used to detect procarcinogens in human cells in culture. However, the activation conditions for each compound must be optimized before comparisons of mutagenic potencies can be made. [This work was performed under the auspices of the United States Department of Energy.]

P-8


BALB/c 3T3 CI A31-I cells (T. Kakunaga, National Cancer Institute) were used to simultaneously assay induction of cytotoxicity, mutation to ouabain resistance (oua<sup>R</sup>) and neoplastic transformation by model chemical toxicants. Cells were incubated for 2 hr with chemical in the presence of a 9000 X g supernatant derived from an Aroclor 1254-induced Fischer 344 rat liver homogenate (S-9) and an NADPH-generating system. The S-9 was characterized as having a total protein content of 17 mg/ml, an aryl hydrocarbon hydroxylase activity of ~300 pmoles 3-OH benzo(a)pyrene (BaP) produced/min/mg protein, was non-toxic to 3T3 cells, and activated model promutagens to metabolites mutagenic to E. coli tester strains. In the presence of a metabolically active S-9 for 2 hr, sensitivity was demonstrated for such chemical classes as polycyclic aromatic hydrocarbons, aromatic amines and mycotoxins: BaP, N-(2-fluorenyl)acetamide (2-AAF) and aflatoxin B<sub>1</sub> (Af B<sub>1</sub>) exhibited both mutagenic and transforming activity; the relative activities were: BaP<sup>2</sup>-AAF<sup>2</sup>-Af B<sub>1</sub>. Structural analogs of these, i.e. benzo(e)pyrene (BeP), N-(4-fluorenyl)acetamide (4-AAF) and aflatoxin G<sub>2</sub> (Af G<sub>2</sub>) were mutagenic only, i.e. BeP> Af G<sub>2</sub>-4-AAF. The oua<sup>R</sup> phenotype was heritable and transformation was confirmed in soft agar and by tumorigenicity. Inactivation of S-9 by heat denaturation reduced or eliminated the cytotoxic, mutagenic or transformation response. Thus the 3T3 system is amenable to the evaluation of multiple biological endpoints induced by a 2 hr exposure to diverse classes of chemical mutagens/carcinogens in the presence of exogenously supplied mammalian metabolizing activity. Supported in part by contracts from the U.S. Environmental Protection Agency.

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Particulate extracts from six different emission sources were assayed for genotoxic activity in mouse BALB/c 3T3 Clone A31-1 cells (T. Kakunaga, National Cancer Institute). All compounds were tested for both transforming and mutagenic potential with and without exogenous metabolic activation in the form of a 9000 x g post-mitochondrial hepatic supernatant fraction from Aroclor-1254 induced Fischer 344 rats. Dichloromethane particulate extracts from the exhausts of two light duty diesel engines, one heavy duty diesel engine, and one late model gasoline engine were assayed in an identical manner to particulate extracts from roofing tar and coke oven emissions. No clear dose responses were observed, but several of the samples showed significant transforming and mutagenic activity. A qualitative ranking system showed the activity of these particulate extracts for either mutagenesis or transformation was: coke oven = gasoline engine > light duty diesel engine #1 > roofing tar. Particulate extracts from light duty diesel engine #2 and a heavy duty diesel engine showed essentially no activity. Supported in part by contracts from the U.S. Environmental Protection Agency.

IN VITRO MUTAGENESIS AND SISTER CHROMATID EXCHANGE EVALUATIONS OF PARTICLE-BOUND ORGANICS FROM COMBUSTION SOURCES. E. L. Evans, M. M. Jotz, and A. D. Mitchell, Biochemical Genetics Department, SRI International, Menlo Park, CA 94025 and J. L. Huisingh and M. M. Moore, Genetic Toxicology Division, Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711

In vitro mutagenesis in the L5178Y mouse lymphoma TK+/- assay system and sister chromatid exchanges (SCE) in Chinese hamster ovary cells were measured in response to eight samples of particle-bound organics from combustion sources. The testing was performed in the absence and in the presence of S-9 from Aroclor 1254-induced Fischer 344 rats, and all testing was performed using coded samples. Upon decoding, the samples were identified as three emissions from diesel cars, one emission from a heavy-duty diesel engine, one from a gasoline car with a catalytic converter, and emissions from cigarette smoke, roofing tar, and a coke oven. All samples were found to induce mutagenesis in the absence and in the presence of S-9 in the L5178Y mutagenesis assay. All samples except one from a diesel Oldsmobile induced significant increases in SCEs in the absence of activation. The emission from a gasoline car was not tested for SCEs in the presence of metabolic activation. Samples from two diesel cars (Nissan and VW), roofing tar, and the coke oven induced significant increases in SCEs in the presence of S-9; the diesel Oldsmobile, the heavy-duty diesel, and the cigarette smoke condensate failed to induce significant increases in SCEs in the presence of S-9. These results suggest that the mixtures from the various combustion sources contain substances that are direct-acting mutagens and that all but one are direct-acting chromosome-damaging agents. (Supported by EPA Contract 68-02-2947.)

DEVELOPMENT OF IN VIVO GERMAL MUTATION SYSTEM USING MONOSPECIFIC ANTIBODY AGAINST SPERM SPECIFIC LACTATE DEHYDROGENASE: SUCCESSFUL DETECTION OF PRESUMPTIVE MUTANT SPERM FROM MICE TREATED WITH PROCARBAZINE. Aftab A. Ansari, Masoor A. Baig,* and Heinrich V. Malling, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.
This paper describes the development of a new method that may be used as an in vivo mutation screening system in mammals. The method utilizes monospecific antibody against lactate dehydrogenase-X (LDH-X), an isozyme of LDH that is present only in testes and sperm. A monospecific antibody that reacts with rat LDH-X but not with mouse LDH-X was prepared from rabbit anti-rat LDH-X antiserum. By the use of this monospecific antibody it was possible to study point mutation in mouse sperm by fluorescent antibody technique. The method for the detection of presumptive mutant sperm using this monospecific antibody is as follows. Mouse sperm are incubated with the rabbit anti-rat LDH-X antibody, washed, and further incubated with fluorescein-conjugated goat anti-rabbit gamma globulin. The unstained sperm represent normal sperm with respect to LDH-X whereas the stained sperm represent sperm containing mutant LDH-X in which an amino acid has been replaced by another amino acid normally present in rat LDH-X at a particular position in the primary sequence. Using this technique 72 'mutant' sperm have been identified among 167 x 10^6 sperm from untreated DBA/2 mice. This value corresponds to a spontaneous mutation frequency of 0.4 x 10^-6. Mice were treated with various doses of procarbazine, a drug that is known to cause mutation in the germ cells. Examination of sperm from procarbazine treated mice showed that the frequency of the 'stained' sperm increased linearly (to 9.2 x 10^-6) with increasing dose of procarbazine from 200 mg/kg to 800 mg/kg. Such a dose vs. response relationship with a known mutagen indicates that the 'stained' sperm are actually mutant sperm in this technique.

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DEVELOPMENT OF IN VIVO SOMATIC MUTATION SYSTEM USING HEMOGLOBIN VARIANTS OF MOUSE: PREPARATION AND USE OF ANTI-S HEMOGLOBIN ANTIBODY IN THE DETECTION OF RED CELLS CONTAINING D HEMOGLOBIN, Masroor A. Baig, Aftab A. Ansari, and Heinrich V. Malling, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

A somatic mutation screening system is described that is based upon the use of an anti-hemoglobin antibody which reacts with Hb-g but not with Hb-d of the mouse. In an attempt to raise anti-Hb antibodies, several horses and rabbits were immunized with Hb-s (from C57BL/6 mouse) and Hb-dmaj (from DBA/2 mouse). The antisera were found to contain high titers of antibodies against the hemoglobins. However, as expected, most of these antibodies were directed against determinants common between s and dmaj hemoglobins; and, therefore, were cross-reacting. Attempts to purify monospecific antibodies from these sera by absorption on Hb immunoabsorbents resulted in poor yield and low affinity antibodies that were inadequate for immunofluorescence work. Subsequently, hemoglobins from C57BL/6 (Hbg), DBA/2 (Hbd), and SJL (Hbg) were injected into 21 groups of mice of various strains differing at the Hb gene loci. The recipient strains included: DBA/2, AU/Sa, CxBL/By, CxBL/By, SEC/1RE, C58, C3HeB/Fe, PL, LP, C57BL/6, C57BR/cd, SJL, Balb/c, SM, and SWR. Out of all these groups, LP mice immunized with C57BL/6 Hb gave an antibody that reacts with C57BL/6 red cells and not with DBA/2 cells. This specific antibody gives strong immunofluorescence and can be used to pick up mutation from d to s hemoglobin in DBA/2 mice. Studies with artificial mixtures containing C57BL/6 red cells in frequencies ranging from 1 x 10^-2 to 1 x 10^-3 showed this antibody can detect rare Hbs-containing red cells in the presence of vast excess of Hbd-containing cells in DBA/2 mice. Examination of amino acid sequences of mouse β chains suggests that this antibody detects point mutations, most likely single base-pair substitutions, at various locations in the Hbb gene locus.
A study of the induction of unscheduled DNA synthesis by physical and chemical agents in non-proliferating primary cultures of rat hepatocytes.

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Viable hepatocytes, isolated by means of an in situ collagenase perfusion of rat livers, were placed in primary culture and used to detect induction of unscheduled DNA synthesis (UDS) by a variety of environmental mutagens and carcinogens. The incorporation of $^3$H-thymidine into DNA due to UDS induced by N-OH-2 acetylaminofluorene (N-OH-AAF), aflatoxin B$_1$ (AFB$_1$), ethyl methanesulfonate (EMS) and ultraviolet light (UV) was quantitated by autoradiography and by scintillation spectrometry on acid precipitable macromolecules or DNA isolated by isopycnic banding in cesium chloride. Dose-dependent increases in UDS due to N-OH-AAF and AFB$_1$ treatment were found. Only 2-fold increases at the highest doses were found, however, when incorporated $^3$H-thymidine was quantitated by scintillation spectrometry. Seven, 11, and 25-fold increases in UDS induced by AFB$_1$, N-OH-AAF and UV respectively, were found when UDS was quantitated by autoradiography, indicating a high sensitivity for detecting "long patch" repair. Scintillation spectrometry was completely ineffective in detecting EMS-induced UDS, whereas autoradiography demonstrated a significant induction at high dose levels. These observations suggest that the sensitivity of the primary hepatocyte/UDS system for detecting DNA damage caused by carcinogens or mutagens which induce "short patch" repair, is limited to autoradiographic analysis.

MUTAGEN-INDUCED CHROMOSOME LESIONS IN LYMPHOCYTES OF SAGUINUS OEDIPUS TAMARINS — A POSSIBLE GENETIC MARKER FOR ANIMALS AT RISK FOR COLON CANCER.

Anne M. Sayer, L. Cayle Littlefield, R. J. Dufrain, and C. B. Richter*, Oak Ridge Associated Universities, Medical and Health Sciences Division, Oak Ridge, TN 37830.

Adenocarcinoma of the colon has been observed at necropsy in 19 of 149 S. oedipus while the disease is not found in members of the related species, S. fuscicollis, housed in the same colony. The high incidence of this malignancy in S. oedipus may have a genetic basis. Since chromosome fragility has been observed in several inherited human syndromes characterized by an increased risk of malignancy, lymphocytes from 10 S. oedipus and 10 S. fuscicollis were exposed to the alkylating chemical mitomycin C (MMC) to determine whether there are species or individual differences in sensitivity to induced cytogenetic lesions. Sister chromatid exchange (SCE) evaluations in a total of 65 lymphocyte cultures showed no difference in either spontaneous or induced SCEs between the two species. However, significant increases in MMC-induced chromosome breaks and SCEs were observed in one S. oedipus, and subsequently in its mother and two siblings, suggesting a familial sensitivity to chromosome lesion induction. Similar increased sensitivity to MMC was observed in cultured lymphocytes of two S. oedipus having cancer of the colon. The observations of increased SCEs in MMC-treated lymphocytes of several members of one S. oedipus family combined with the observation of increased sensitivity to induction of MMC lesions in two non-related animals with colon cancer suggest that increased chromosome sensitivity may be a genetic marker for those tamarins at risk for cancer of the colon. (Supported by USDOE Contract No. DE-AC05-76RO00033, and NIH Grants HD08828 and 5P40 RR 01125 02 PME.)
NONDESTRUCTIVE CYTOGENETIC TOXICOLOGIC TESTING WITH RATS. Patricia A. Beltz* and R. Daniel Benz (intr. by Sheldon A. London), University of California, Irvine - Toxic Hazards Research Unit, P. O. Box 3067, Overlook Branch, Dayton, Ohio 45431.

To validate two short term in vitro tests that look for induced cytogenetic damage in mammalian cells, we have used methods developed for obtaining tissue from rats, the classical model for toxicologic testing, without the necessity for sacrificing any animals. Because no animals are killed, the effect of chemical exposures can be compared between the results of short term in vitro tests and long term, classical toxicologic tests using the same animals for both kinds of tests. This method of validation eliminates problems that occur when results of different laboratories using different procedures are compared. We also can analyse serial samples from the same animals during chronic exposures with these methods. Finally, our methods can be readily applied directly to humans for possible diagnostic or prognostic use. The specific short term tests we are using are the sister chromatid exchange (SCE) test, a very sensitive assay for induced mammalian cytogenetic damage, and the micronucleus test, a very quick and simple assay for induced chromosome breakage. We are currently doing these tests with rat peripheral lymphocytes and mixed bone marrow cells. So far, we have done in vitro tests with 1,1-dimethylhydrazine (UDMH) and, as a positive control, mitomycin C (MMC). Both were found to induce SCEs and micronuclei in primary rat peripheral lymphocytes and MMC was found to be capable of inducing SCEs in primary rat bone marrow cells. Animals will soon be treated in vivo with these chemicals and results analyzed.

THE EFFECT OF AROMATIC AMINES ON SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS. Lisa McConlogue*, Alisa L. Katzen*, June J. Andersen, and Ann D. Burrell, IBM Corporation, 5600 Cottle Road, General Products Div., San Jose, CA, 95193.

The effects of 3 aromatic amines were examined for their ability to induce sister chromatid exchanges (SCEs) in cultured Chinese hamster ovary cells, both in the presence and absence of liver microsomal enzyme activation (S9). Benzidine, a known carcinogen, gave a dose response curve with 0.72-0.8 SCEs/chromosome at 2 x 10^-8 M in the absence of S9, and a similar or slightly reduced level in the presence of S9. This indicates that the cells still retain some metabolic activity and can metabolize benzidine to its active component. The non-mutagenic derivative 3,3',5,5' - tetramethylbenzidine gave values of 0.4 SCE/chromosome both with and without S9 activation at all concentrations tested. These values are not significantly different from background values indicating good correlation between known mutagens/ carcinogens and the capacity to induce SCEs. When N,N,N,N',N'-tetramethylbenzidine (a mutagen) was tested in this assay, it proved to be highly insoluble at the levels at which SCEs were observed with benzidine. At concentrations at which it was soluble, no increase in the number of SCEs was observed. 4-nitroquinoline oxide, also an aromatic amine, was included as the positive control and a dose response curve was obtained.
SISTER CHROMATID EXCHANGES IN VIVO IN MICE: SYNERGISTIC INTERACTION BETWEEN TRIETHYLENEETHAMINE AND BROMODEOXYURIDINE, J. L. Wilmer and E. R. Soares, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

The increased sensitivity of bromodeoxyuridine (BrdUrd)-substituted DNA to physical agents such as X-ray and UV light is well-documented. However, only two recent reports have addressed this problem with regard to chemical mutagens and the potential for increased interaction with BrdUrd-labeled DNA using sister chromatid exchange (SCE) as the endpoint. The possibility of a synergistic interaction between BrdUrd and TEM, a potent polyfunctional alkylating agent, was investigated. BrdUrd pellets (30 or 50 mg) were implanted subcutaneously in strain DBA/2J male mice. A single ip dose of TEM was administered 8 hr. post-implantation at either 1.25, 6.25, 12.5, 62.5 or 125.0 μg/kg. Demecolcine (1.4 mg/kg) was injected ip at 21 hr. post-implantation to arrest cells in metaphase. Two hours later mice were killed and their femoral bone marrow was prepared following standard cytogenetic protocols. After processing through the fluorescence-plus-Giemsa technique, differentiated second division cells were analyzed for SCEs and chromosome aberrations. A consistent dose response for chromosome aberrations was not found at these low doses. However, TEM induced significant dose-related increases in SCE frequencies. The control SCE frequencies for the 30 and 50 mg pellets remained relatively constant (5.7 – 6.6 SCE/cell) while the frequencies were approximately 3.7 – 6 fold higher with the high dose of TEM. The slope of the dose response curve for the 50 mg pellet was significantly different from the 30 mg pellet. This differential induction rate appeared to be synergistic, suggesting that BrdUrd-substituted DNA is more sensitive to the action of TEM.

CYTOGENETIC EFFECTS OF cis-PLATINUM(II)DIAMINEDICHLORIDE (cis-PDD) ON RABBIT AND HUMAN CELLS. W. D. Morrison, V. Huff, S. Colyer,* C. Littlefield, and R. DuFrain. Oak Ridge Associated Universities, Medical and Health Sciences Division, Oak Ridge, TN 37830.

The rabbit has been proposed as a model for assessing the risk to humans of exposure to chemical mutagens. Our purpose was to study the effects of the chemotherapeutic agent cis-PDD on human and rabbit lymphocytes in vitro and on rabbit marrow cells (BM), lymphoblasts (LN), and lymphocytes in vivo. Blood cultures from 4 rabbits and 2 humans were treated with cis-PDD. Cells exposed in G0 or G2 were analysed for sister chromatid exchange (SCE) and/or chromosome aberrations. For in vivo studies, 4 rabbits were given intravenous injections of cis-PDD. Post-treatment blood samples were withdrawn for analysis and rabbits were sacrificed at 6 hours and 24 hours for BM and LN cytogenetic analysis. SCE analysis of over 350 human and rabbit metaphases from lymphocytes treated in vitro shows that rabbit lymphocytes are more sensitive to SCE induction by cis-PDD. Significant numbers of SCE appeared in cultures obtained as early as 1 hour post-treatment in lymphocytes from injected rabbits. No significant increase in chromosome aberrations was observed in 500 human and rabbit metaphases from cells treated in vitro in G0 or G2 with cis-PDD or in lymphocytes from rabbits treated in vivo. Analysis of 800 LN and BM metaphases from rabbits treated in vivo showed a high percentage of abnormal metaphases in these proliferating cells. No difference in sensitivity was observed, but BM showed a delayed response to treatment with cis-PDD. These data indicate that cis-PDD is an effective clastogen in proliferating cells and produces lesions which lead to SCE in non-proliferating cells. (Supported by U. S. Department of Energy Contract No. DE-AC05-76OR00033 and NICHD Grant #HD-08828.)
INDUCTION OF SPECIFIC LOCUS MUTATIONS AND SIBLING ("SISTER") CHROMATID EXCHANGES BY BROMODEOXYURIDINE IN CHINESE HAMSTER OVARY CELLS.
J. S. San Sebastian, J. P. O'Neill, A. Johnson* and A. W. Hsie, Univ. of Tenn.-Oak Ridge Graduate School of Biomedical Sciences, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

The relationships among BUdR incorporation by CHO cells, the induction of 6-thioguanine resistant mutants (CHO/HGPRT system) and sibling chromatid exchanges (SCE) was investigated with BUdR concentrations in the range of 0.1-200 μM and a 25 h incubation period. The incorporation of radio-labeled BUdR into precipitable macromolecules (i.e., DNA) increased linearly with concentration up to 5-10 μM; at higher concentrations, the incorporation appeared to plateau. The induction of mutations was found only at concentrations greater than 50 μM, and induced frequencies of approximately 2000 mutants/10^6 survivors were obtained. The frequency of SCE increased linearly with BUdR concentration over the testable range of 1-100 μM. These observations suggest that the induction of SCE by BUdR is a result of BUdR incorporation into the cellular DNA, but that both SCE and mutations are induced by a mechanism other than incorporation.

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SISTER CHROMATID EXCHANGE IN HUMAN FIBROBLAST-RAT HEPATOCYTE CO-CULTURES.
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One of the major difficulties in using an in vitro system for the detection of genotoxic agents is the need to account for the biochemical reactions performed by the mammalian liver. In many studies a mammalian microsomal fraction is added to bacteria or cells in culture in an attempt to mimic the hepatic metabolic processes. To approximate more closely an in vivo situation, we have chosen to use whole isolated rat hepatocytes to metabolize procarcinogens. Our approach is to culture rat parenchymal hepatocytes with human fibroblasts and monitor sister chromatid exchange (SCE) in the fibroblasts. The livers of male Sprague-Dawley rats were perfused with 0.5mg/ml collagenase solution and the hepatocytes isolated and plated over confluent human fibroblasts. After allowing four hours for hepatocyte attachment, either cyclophosphamide (CP) or diethylnitrosamine (DEN) were added in serum-free media. Twenty hours later the cells were trypsinized and replated in media containing 20μM BrDU for two cell cycles. Initial studies with 0.5μg/ml of CP showed that when 15 to 20x10^6 hepatocytes were plated over confluent fibroblasts in 100 mm tissue culture dishes, attachment and spreading of the hepatocytes were good, and few dead cells or uncovered fibroblasts were seen. Increasing the number of hepatocytes from 5 to 50x10^6 cells/dish had no significant effect on SCE rates. When increasing doses of CP (0.125 to 2.0μg/ml) were added to the co-cultures, a concomitant linear increase in SCE rates was observed. Fibroblast cultures without hepatocytes exposed to identical doses of CP failed to show elevated SCE rates. In contrast to CP, DEN failed to induce a significant increase in SCE at doses as high as 20mM. These results parallel published in vivo studies with CP and DEN, and show that hepatocytes can deliver long-lived active metabolites to fibroblasts in vitro.
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INDUCTION OF SISTER CHROMATID EXCHANGES BY CIGARETTE SMOKE CONDENSATE FRACTIONS WITH OR WITHOUT S-9. W.F. Benedict, J.A. Dawson* and A. Banerjee* Division of Hematology-Oncology, Department of Medicine, Childrens Hospital of Los Angeles and Department of Pediatrics University of Medicine, Los Angeles, Calif. 90027.

Cigarette smoke condensate (CSC) fractions, the whole condensate and reconstituted condensate were tested for their ability to produce sister chromatid exchanges (SCEs) following a 1 or 2 hr exposure in the presence or absence of S-9 using three different mammalian cell types. The fractions, NNM, Bpb and WAI, produced a greater than two-fold increase in SCEs after a 1 hr exposure and was concentration dependent. Only fraction NNM, however, required S-9 to cause this increase in SCEs. Fractions Bpb and WAI had previously been found to be particularly mutagenic in the Salmonella typhimurium assay (Kier et al. Proc. Natl. Acad. Sci. 71:4159, 1974) and had been shown by us to produce malignant transformation (Benedict et al. Cancer Res., 35:857, 1975). The mutations obtained by fractions Bpb and WAI in Salmonella, however, required S-9. Thus, it is likely that different chemicals in these CSC fractions are responsible for the mutagenesis in Salmonella and the increases in SCEs reported here. In addition, fraction NNM (which contains the known carcinogen benzo[a]pyrene) was not mutagenic in the Salmonella assay although it yielded a considerable increase in SCEs. Thus, the production of an increase in SCEs may be a highly sensitive indicator of those CSC fractions which contain potential hazards to man.

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TENTATIVE IDENTIFICATION OF CHO-UV-1 AS A UV-SENSITIVE MUTANT DEFICIENT IN POST-REPLICATION REPAIR. L.L. Hinkle*, C.A. Waldren & T.D. Stamato*. Eleanor Roosevelt Institute for Cancer Research; Dept. of Biochem., Biophys. & Genetics, Univ. of Colo. Health Sciences Center, Denver, CO 80262 & the Wistar Institute of Anatomy & Biology, Phila., PA 19104.

CHO-UV-1, a mutant derived from the Chinese hamster ovary cell (CHO-K1) by nylon cloth replica plating, is hypersensitive to killing by ultraviolet light [TDS & CAW (1977) Somatic Cell Genetics 3:431], ethyl methanesulfonate (EMS) and N-methyl-N'-nitroso-N-nitrosoguanidine (MNU), but has normal resistance to X-ray and 4-nitroquinoline-1-oxide (4NQO). Caffeine enhances the lethal action of these agents with which it has been tested: UV, EMS, and X-ray. Fusion of UV-1 with itself produced pseudo-hybrids still hypersensitive to UV. Hybrids of UV-1 and Chinese hamster lung cells (CHL) had normal resistance. The mutation in UV-1, therefore, appears to be recessive. Analysis of UV-1 by radiographic and alkaline lysis-hydroxyapatite techniques indicates that UV-induced excision repair is not significantly diminished. The conversion of low molecular weight DNA into large size molecules by UV-irradiated cells is 6-fold slower in UV-1 than in the parent, indicating a deficiency in UV-1 in post-replication repair. When the frequency of EMS-induced mutations for UV-1 and its parent are compared as a function of survival, UV-1 is approximately 3-fold less mutable. Caffeine decreases the mutation frequency for the parental cell, but does not alter that of UV-1. We have interpreted these data to show that UV-1 has a mutation in post-replication repair, and that this system is responsible for a large proportion of the mutants seen in mutagenized normal cells. (Contrib. No. 311 from ERICR. Supported by USPHS ES02273, ES0155 and Elsa U. Pardee Foundation.)
POSTREPLICATION REPAIR IN YEAST  M. A. Resnick, J. Bryce, B. Cox, intr. by J. Drake, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, and Oxford University, Oxford, England

Exposure of yeast to ultraviolet light (UV) greatly enhances genetic recombination; the relevant lesions in the absence of excision repair are pyrimidine dimers. We have examined whether or not the molecular mechanism of exchange observed for Escherichia coli, wherein postreplication repair is associated with the recombination of DNA regions containing pyrimidine dimers, exists in yeast. As shown by di Caprio and Cox (submitted for publication) postreplication repair occurs in Saccharomyces cerevisiae following low UV doses (<4 J/m²). Various haploid and diploid excision-defective mutants (rad 1, rad 3, and rad 4) were grown overnight in 3H uracil--irradiated--chased for 45 minutes--pulse labeled with 14C uracil for 60 minutes--and chased for 2 hours. The UV-endonuclease enzyme activity in Micrococcus luteus extracts was used to examine whether pyrimidine dimers induced in parental DNA strands became associated with daughter strands. To enhance sensitivity, a method was developed in which full-length chromosomal DNA could be treated with the extract; the assay was sensitive to doses below 0.5 J/m². Based on this assay system, there is no exchange of pyrimidine dimers after 2 or 4 J/m² in either haploid or diploid excision-defective mutants of yeast.


Because of the importance of DNA repair in mutagenesis, we are isolating repair-deficient mutant lines, on the basis of their hypersensitivity to UV radiation or chemical mutagens. Using CHO cells, we obtained more than 45 stable UV-sensitive strains and several strains that are sensitive to alkylating agents (but not UV). A screening procedure was devised to identify quickly each new complementation class. Cultures of UV-sensitives were mixed in pairs, fused with 45% polyethylene glycol, replated, and irradiated with a fluence of UV (5 J/m²) that kills mutant cells but not wild type (WT). Complementation is indicated by frequencies of colony formation that significantly exceed the backgrounds obtained in self-cross control cultures. The results obtained to date on 45 UV sensitives have identified at least 3 genetic classes, probably 4 (preliminary), as follows: UV class 1, 4 strains; class 2, 38; class 3, 2; class 4, 1. One class 2 mutant was derived in an EMS-sensitive strain, resulting in a phenotype hypersensitive to a wide variety of mutagens. ICR-170 was used to induce most of the strains, but some members of classes 1 and 2 were also obtained with EMS. The recessivity of UV-sensitive phenotypes in classes 1-3 was shown by the survival curves of tetraploid hybrids made using drug-resistance markers. Similarly, complementation between mutants of classes 1 and 2 was explicitly shown with hybrids; their survival curves matched that of WT. Supported by U.S. DOE contract W-7405-ENG-48 to LLL and grant nos. LBL 71347090 (DOE) and GM-2202L and RR 00961 (NIH) to UCB.

5-bromodeoxyuridine (BrdU) tablet implantation is a technically simple in vivo labelling technique which has been used in rodents to examine tissue-specific sister chromatid exchange (SCE) induction by chemical mutagens. We have extended the scope of this approach to include analyses of embryo tissues after exposure to teratogens and transplacental carcinogens. Maternal mice or rats were subcutaneously implanted with a BrdU tablet and subsequently injected ip. with cyclophosphamide, procarbazine, or 4-nitroquinoline 1-oxide. Significant increments in SCE were detectable in embryonic liver and maternal bone marrow. Additional embryonic tissues analyzable for SCE included lung and yolk sac. Under conditions of similar analogue substitution, rat yolk sac was studied after in vivo (maternal) or in vitro exposure to cyclophosphamide. At day 11 of gestation, maternal dosing with 5, 10, and 20 mg./kg. resulted in the transplacental induction of SCE at levels approximating 2- to 4-fold respectively over control. In some instances, BrdU-substituted conceptuses were explanted to whole-embryo culture conditions where they were treated with up to 100 µg./ml. of cyclophosphamide. DNA synthesis and cell cycling progressed in the absence of SCE induction. Conceptuses alternatively cultured with 0.05 or 0.50 µg./ml. phosphoramid mustard, a presumed metabolite of cyclophosphamide, revealed approximately 2- and 6-fold elevations respectively in SCE. Thus, SCE induction in embryonic tissues may be assessed after transplacental or direct chemical exposures.

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THE EFFECT OF PRIOR PARTIAL HEPATECTOMY OR CO-ADMINISTERED TOLUENE ON BENZENE-INDUCED BONE MARROW DAMAGE. R.R. Tice, D.L. Costa and R.T. Drew, Medical Department, Brookhaven National Laboratory, Upton, NY 11973.

Studies were initiated to examine the relation between general metabolism or liver-specific metabolism and benzene-induced bone marrow damage (as measured by an increase in sister chromatid exchange (SCE) frequencies). DBA/2 male mice (~3 mo. of age) were exposed to benzene (3000 ppm-4 hr) one day after partial heptectomy or in the presence of toluene injected ip. Loss of two-thirds of the liver had no effect on benzene-induced SCE frequencies (benzene exposed animals had a mean of 24.5 + 2.6 (S.E.M.) SCE/cell, partial heptectomized animals had a mean of 25.6 + 1.7 SCE/cell). Toluene (28 mM/kg in corn oil) administered ip. immediately prior to benzene inhalation decreased the yield of SCE by 52%. Since toluene appeared to adversely affect breathing rates, some animals were treated with both benzene and toluene injected ip. Using a concentration of benzene equal in effect (as measured by the level of induced SCE) to the inhaled concentration, toluene co-administered at equal molar concentration (18.8 mM/kg) depressed the ability of benzene to induce SCE by 91%. Since toluene is a well-known competitive inhibitor of benzene metabolism in viva, these results indicate that while metabolism of benzene is a necessary process before genetic damage occurs in bone marrow cells, the liver may not be the main site of production for the metabolite capable of inducing SCE.

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The Effect of Biotransformation of 2,4-Dinitrotoluene on Its Mutagenic Potential. D. B. Couch, D. J. Abernethy, P. F. Allen and D. L. Ragan.* Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, NC 27709.

Since both oxidative and reductive metabolism of the hepatocarcinogen 2,4-dinitrotoluene (2,4-DNT) can occur in vivo, we have examined the mutagenicity of compounds which can be formed from 2,4-DNT in an attempt to establish which metabolic pathways contribute to the formation of
genotoxic products. The Ames Salmonella/microsome test and a quantita-
tive reversion assay using S. typhimurium TA98 were used to evaluate the
mutagenicity of these compounds. On a molar basis, 2-amino-4-nitrotolu-
ene and 2-nitro-4-aminotoluene are up to 20-fold more mutagenic to S.
typhimurium TA98 than is 2,4-DNT and do not require metabolic activation
by post-mitochondrial supernatants of Aroclor-induced rat liver homogen-
ates (PMS) for their effect. 2-Amino-4-nitrobenzoic acid is also more
mutagenic than 2,4-DNT to Salmonella, particularly with added PMS. 2,4-
Dinitrotoluene, a minor metabolite in vivo, requires PMS for demonstra-
tion of mutagenicity and is approximately as effective, on a molar basis,
as 2,4-DNT in inducing reversion to histidine protrophy. 2,4-Dinitro-
benzyl alcohol produced 2-3 fold increases in mutant fraction over those
induced by equimolar concentrations of 2,4-DNT. While products arising
from both oxidative and reductive metabolism show increased mutagenicity
to Salmonella relative to the parent compound, aminonitro compounds were
the most potent mutagens of the metabolites studied. These results
suggest that reduction of aromatic nitro groups of 2,4-DNT may be es-
specially important in formation of genotoxic products in vivo.

The generation of phenotypic dimorphism in cells of Bloom's
syndrome: Somatic recombination as a mechanism. A.B. Krepsky*
and J.A. Heddle, York University, Downview, Ont., Canada.
Bloom's syndrome (BS) is a rare disease inherited by an
autosomal recessive mode. The characteristics of the disorder
include abnormally high frequencies of sister chromatid ex-
changes (SCEs), elevated rates of chromosomal aberrations, and
hypersensitivity to EMS. Among the chromosomal aberrations,
the symmetrical quadriradial configurations (Qr's), that re-
sult from an interchange between two homologous chromosomes,
are the most typical ones. They have been previously presented
as evidence for mitotic crossing-over. Some individuals aftec-
ted with BS have two populations of lymphocytes circulating
in their blood: one with high spontaneous rate of SCEs, char-
acteristic of BS (high cells), and one with low rates, simi-
lar to those in normal individuals (low cells). The high cells
are hypersensitive to EMS, whereas the low cells have normal
sensitivity. We propose a somatic recombination model as a
possible explanation for this phenomenon. The model is based
on an assumption that two linked loci are responsible for BS:
a structural locus bls, which codes for an enzyme, and a regu-
ulatory locus blr, which controls the rate of the enzyme syn-
thesis. The blr gene is assumed to exercise its control over
the bls gene in the cis position only. Thus at least three
genotypes of BS can exist. Only one of them, namely double
heterozygotes with mutated bls gene in one chromosome and mu-
tated blr gene in its homologue, would generate low cells by
somatic recombination via the Qr's that characterize the con-
dition.

THE MICRONUCLEUS ASSAY: TESTING WITH AN IMPROVED PROTOCOL. Michael F.
Salamone and John A. Heddle, Dept. Biology, York University, Downview
(Toronto), Ontario, Canada
Time-effect studies with the drugs mitomycin C, cyclophosphamide, and
dimethylbenzanthracene indicated that the time course of micronucleus
production in PCE was different for each of these chemicals. Based upon
these and other results, we have developed a modified micronucleus protocol
which employs multiple sample times rather than multiple doses. In the
first phase, animals are injected intraperitoneally at 0 and 24 h with the maximum tolerated dose (MTD) and sampled at 48, 72 and 96 h. If a negative result is obtained, then a second phase is initiated in which animals are injected at 0 h with ½ the MTD and sampled at 30, 48 and 72 h. In each case, a positive result can be confirmed by either a repeat experiment or a dose-effect study at the time of the maximum response. Our current practice is to score 500 PCE/mouse and 3-6 mice per sample. The frequency of mice, in our cumulative control samples, which have 0, 1, 2,3 etc. spontaneous micronuclei per 500 PCE is adequately described by the Poisson distribution. It is easy, therefore, to choose our criteria for a positive response so that the accepted level of false positives in any single experiment is set at 10%. Therefore, the probability of a false positive in two successive experiments would be 1%. Since false positives which are confirmed can also arise among those groups in which the first and second experiments do not agree and a third is used for a decision, the actual accepted false positive level is ~ 3%. Although there may be other improvements that can be made, our results suggest that the above protocol will detect a higher proportion of mutagens than protocols that utilize only sampling times of about one day.

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Results from our laboratory as well as others (e.g. Commoner, Pariza, Springarn) have shown that the organic basic fraction from grilled hamburger (BFH, in g equiv. wet wt of meat) is highly mutagenic in Salmonella (3700 revertants/10^8 TA 1538 cells/40 g BFH/ml top agar). The significance of these results is being evaluated with short-term mammalian bioassays. BFH (3 g/ml) induced equal toxicity in mouse hepatoma cell lines that differ in aryl hydrocarbon hydroxylase activity. In CHO cells BFH (45 g/ml) induced cytotoxicity with or without S9.

Sister chromatid exchanges (SCE) in bone marrow cells were not significantly increased in C57BL/6J mice exposed to BFH at 1.0, 0,1 and 0.03 x LD50 (22.5 g BFH/g body wt). The BFH injected simultaneously with BaP did not mask the positive SCE response to BaP. Juvenile female mice exposed intraperitoneally to BFH were examined for destruction of primordial oocytes. There was no statistically significant difference between mice treated with concentrations as high as the LD50 and controls injected with corn oil. Thus, BFH has shown cytotoxicity in cultured cells, but no significant genotoxic effects in in vivo mammalian assays at concentrations that produce significant mutations in Salmonella. Work performed under the auspices of the US Department of Energy contract no. W-7405-ENG-48 and National Institute of Environmental Health Sciences interagency agreement 2240L-ES-80038.

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We have developed an improved extraction procedure for mutagens in cooked foods and tested for mutagenicity in five Salmonella strains. Reports by Commoner, et al., Pariza, et al., and Springarn and Weisburger are confirmed that bacterial mutagens are formed during grilling of ground
beef at 200°C. In the basic fraction an average of 3700 revertant colonies in 10^8 cells of strain TA 1538 /100 g of hamburger was obtained by acetone extraction, nearly a two-fold increase over 2000 revertants by the original procedure with acidic ammonium sulfate. The mutagenic activity was highly specific for the frameshift-sensitive strains TA 1537, TA 1538, and TA 98, but was absent in the base pair substitution-sensitive strains TA 1535 and TA 100. The mutagens from beef require enzymatic activation, which shows considerable specificity as to species and inducer. The S9 fraction from hamster liver induced by PCB gave the highest yield of revertants (8000/100 g beef). Microsomal fractions from C57BL/6J mice induced by PCB, 3-MC and BNF were more active than fractions induced by phenobarbital or a steroid, or uninduced. The data show specificity for cytochromes P-450, but the species and inducer patterns differ from those of characterized arylamine carcinogens such as 2-AAF and 2-aminoanthracene. Short-term bioassays in mammalian systems are in progress to evaluate the spectrum of genetic toxicity of mutagens from cooked foods. Work sponsored by U.S. Dept. of Energy under Contract W-7405-ENG-48 and National Institute of Environmental Health Sciences interagency agreement 222X01-ES-80038.

P-32
A MODIFICATION OF THE AMES TEST WHICH ALLOWS FOR CALCULATION OF TRUE MUTANT FREQUENCIES. E.J. Greene and M.A. Friedman, Corporate Medical Affairs, Allied Chemical Corporation, Morristown, NJ 07960.
The standard Ames plate incorporation test and liquid preincubation assays were found to yield results which did not allow for the calculation of true mutant frequencies. The reason for this problem was that the trace amount of histidine in the selective medium allowed for growth on the plate such that the revertant number, spontaneous or induced, was relatively independent of the number of bacteria plated. Therefore, a modification of these assays was developed from which true mutant frequencies could be calculated. In this assay the bacteria, test chemical, and, when desired, an activating system were incubated in liquid suspension under growth supporting conditions. Following treatment, the bacteria were washed and further incubated in growth medium for several generations. The treated populations then were washed and plated onto minimal agar in the complete absence of histidine to determine revertant number, and onto non-selective medium to determine total bacteria. From these data true mutant frequencies could be calculated. This assay efficiently detected the mutagenic activity of a variety of mutagens, both direct-acting and requiring metabolic activation. The number of revertants detected was linearly related to the number of bacteria plated, with slopes for log-log plots close to one. This assay has the advantages of a) expressing results as mutant frequencies, instead of revertants per plate; b) allowing recovery from initial toxicity so that highly toxic chemicals or mixtures can be more accurately tested; c) being flexible as to manipulations during the treatment period; and d) being easily modified for testing gases and volatile liquids. Its main disadvantage is that it is more time-consuming than other assays.

P-33
Liver microsome fractions from both male and female Syrian golden hamsters or male and female Sprague-Dawley rats were prepared from untreated animals or animals stimulated with either phenobarbital or Aroclor 1254. The resulting S9 mixes were compared for their abilities to activate the nitroso compounds to mutagenic forms using the Salmonella/
mammalian-microsome mutagenicity test. Tester strain TA1530 was used throughout in the plate incorporation assay. Equal microgram doses of each compound were screened against several different protein concentrations for each microsomal preparation. Dimethyl- and diethylnitrosamine were activated by all the hamster homogenates but were not mutagenic with any of the rat homogenates. Dimethylethynitrosourea and diethylnitrosourea were activated only by induced hamster S9 but not by any rat S9 preparation. Nitrosomethylurea did not require metabolic activation to show mutagenicity and was not inactivated by any of the S9 preparations. Nitrosomethylaniline, an esophageal carcinogen, and diphenylnitrosamine, a bladder carcinogen, were not activated by either hamster or rat preparations. The use of hamster liver homogenates greatly increased the sensitivity of the Ames plate incorporation assay for detecting several carcinogenic nitroso compounds.

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P-34
UV MUTATIONAL SPECIFICITY OF THE DRUG RESISTANCE PLASMID pKM101.
R. G. Fowler, L. McGinty, L. Komar, and K. Mortelmans. San Jose State University, Department of Biological Sciences, San Jose, CA 95192, and SRI International, Life Sciences Division, Menlo Park, CA 94025.

The drug resistance plasmid pKM101 is capable of enhancing both spontaneous and induced mutation frequencies in E. coli and S. typhimurium. Because of its ability to enhance chemical mutagenesis, plasmid pH101 was introduced into certain of the tester strains used in the Salmonella/microsomal chemical mutagenesis assay. Very little is known about the molecular mechanism(s) involved in the plasmid-mediated enhancement of mutagenesis. We have determined the molecular specificity of UV-mutagenesis in E. coli strains with and without pKM101 utilizing the trpA system. Yanofsky and co-workers have determined amino acid changes corresponding to many of the trpA point mutants and revertant classes. These data, when combined with the known structure of the genetic code, permit the deduction of codons at most mutant and revertant sites. The base-pair revertant pattern of a given trpA allele can often be ascertained directly from simple physiological tests. We find that pKM101 trpA strains have a different reversion spectrum of UV-induced Trp+ revertants compared to isogenic strains without pKM101. For example, GC→AT transitions at the suppressor site of trpA58 are strongly enhanced by UV exposure in the absence of pKM101. However, in the presence of pKM101, this reversion event is not further increased whereas AT→CG transversions at the trpA58 site are enhanced. We conclude that pKM101 does not merely enhance cellular SOS error-prone repair, but is associated with an additional repair system(s).

P-35
APPLICATION OF MUTAGENICITY TESTING TO DETECT MUTAGENS IN HUMAN URINE.

We have applied the Ames Salmonella test system for standardizing in vitro techniques to detect the presence of mutagens in human urine. In an attempt to ensure that we were working with urine containing detectable mutagens, we obtained urine from cigarette smokers. We further increased the probability of positive results by collecting daytime and nighttime urines separately because others have reported greater concentrations of mutagens in urine collected during the daylight hours (Yamasaki and Ames, PNAS, 74:3555, 1977). Between 100- and 500-ml aliquots of urine were examined after concentration by XAD-2 (1 g) column treatment, methanol elution,
and subsequent evaporation to dryness. Mutagenic activities in final DMSO extracts were determined using Salmonella strains TA98 and TA100. No net mutagenic activity was detected in any sample without added microsomal S9 fraction, whether or not β-glucuronidase was added. Urine concentrates from cigarette smokers were consistently positive with TA98, but negative with TA100. Increased sensitivity for detecting mutagens was attained by increasing the urinary aliquots analyzed from 100 to 500 mL and increasing the XAD-2 resin from 1 to 5 grams. We have used urinary creatinine concentration as the reference point for comparing both inter- and intrasubject variations in the amount of urinary mutagens excreted. All inhaling cigarette smokers excreted mutagenic urine during daylight hours regardless of the number of cigarettes smoked. Urine from non-inhaling pipe smokers, and non-smoking omnivores and vegetarians was consistently negative.

This work was supported in part by the Diet, Nutrition, and Cancer Program, NCI Contract N01-CP-83620.

P-36

NIRIDAZOLE AS A MUTAGEN: ACTIVATION BY BACTERIAL BUT NOT MAMMALIAN NITROREDUCTASE. L. W. Meyer*, J. L. Blumer*, and W. T. Speck, Department of Pediatrics, Case Western Reserve University, Cleveland, OH, 44106.

The anthelmintic agent niridazole (NDZ), a nitrothiolimidazolidinone, was studied as a model compound in order to evaluate the relative importance of bacterial and mammalian nitroreductases in the metabolic activation of nitroheterocycles. In the standard Ames assay, Salmonella tester strains TA-98, TA-100, and TA-1538 all showed dose-dependent conversion to histidine independence at concentrations of NDZ from 0 to 1 μg/plate. At higher concentrations significant bacterial killing was noted. Similar experiments performed under anerobic conditions resulted in a 2-3-fold increase in the numbers of revertants. Addition of rat liver S-9 with cofactors did not enhance the response either in the presence or absence of O2. To further test the importance of the aromatic nitro-group, a desnitro-NDZ analog was synthesized. This compound was not mutagenic in any of the three Salmonella tester strains even at concentrations exceeding 1 μg/plate. When mutant tester strains grown in the presence of low levels of NDZ were examined for NDZ sensitivity and nitroreductase activity, neither the TA-98, TA-100 nor the TA-1538 could be mutagenized by the drug at concentrations exceeding the LD 90 for their respective wild-type strains. These results suggest that the mutagenicity of NDZ is dependent upon metabolic activation of its aromatic nitro group. Further, bacterial nitroreductases, but not rat liver nitroreductases, were capable by catalyzing these reactions. Caution must therefore be exercised in extrapolating the results of the Ames assay with nitroheterocycles to mammalian systems.

P-37

GENETIC TOXICOLOGY OF SUBSTITUTIONALLY INERT TRANSITION METAL COMPLEXES. G. R. Warren, P. N. Schultz*, E. H. Abbott* and S. J. Rogers, Montana State University, Bozeman, MT 59717

A number of octahedral substitutionally inert complexes of rhodium (III), chromium (III), cobalt (III), iridium (III) and platinum (IV) have been tested for their genetic toxicology using a differential lethality assay on selected repair deficient Escherichia coli K-12 strains and by histidine reversion in Salmonella strains. The nature of the ligands, charge and stereochemistry of the complex profoundly affect the genetic toxicology of these complexes. Chemical and structural features of the rhodium (III) complexes which enhanced DNA damaging capability were a plus one charge on the complex, two adjacent relatively labile groups such as chloride or bromide with four more strongly bonded ligands such as alkyl or arylamines. The structural and stereochemical requirements
for genetically active complexes of chromium (III), cobalt (III), iridium (III) and platinum (IV) do not appear to follow the pattern of ligand requirements demonstrated for the genetically active rhodium complexes but are dependent on the transition metal in the complex. The genetic activity of certain chromium complexes suggest that chromium (III) as well as chromium (VI) can act as the ultimate mutagen. A consistent parallel between mutagenicity in the Ames test and differential lethality in the E. coli K-12 repair assay was demonstrated with all complexes. There was also a consistent requirement for the presence of the pKM101 plasmid in Ames' strains for the demonstration of mutagenicity.

P-38
Two methods for testing gases and volatile liquids for mutagenic activity in Salmonella are compared. Bacteria in semisolid agar or broth were exposed in sealed containers. The agar exposure test was performed by placing lidless plates containing bacteria with or without a rat liver activation system in 9 liter glass chambers. Gas or volatile liquid was introduced and the chambers sealed and incubated for 48 hr at 37°C. Gas concentrations within the chambers were monitored shortly after filling and before emptying by analyzing samples with a gas chromatograph. Vinyl chloride, vinylidene chloride, methylene chloride, Freon® 11, Freon® 12, Freon® 22, and fluorocarbon 31 were tested. All compounds except Freon® 11 and Freon® 12 exhibited reproducible, concentration-related mutagenic activity. The liquid suspension protocol was used to test all compounds except Freon® 11 and Freon® 12. Flasks containing bacteria in nutrient broth with and without an activation system were flushed with gas-air mixtures or treated with volatile liquids, sealed with rubber septa, and incubated with agitation at 37°C for 2 hr. Gas concentrations within the flasks were monitored just prior to the end of treatment. After incubation, cells were collected by centrifugation, resuspended in histidine-free minimal broth and plated in biotin supplemented media. Toxicity was determined concurrently by plating bacteria in histidine containing agar. After incubation at 37°C for 48 hr, revertants/10⁸ survivors were determined. The responses observed with the two test methods were similar for all test chemicals except Freon® 22. Care must be taken in choosing the appropriate test protocol for evaluating mutagenic potential in the Ames Salmonella strains.

P-39
We have designed a closed, inert incubation system for bacterial plates that allows direct head space vapor samples to be withdrawn. Vapor-phase and liquid phase concentrations of volatile chemicals have been determined by gas-liquid chromatography. The properties of this containment system have been investigated using carbon-14 labelled dichloromethane as the test chemical. We have determined the mutagenic potency of ten common halogenated alkanes using the Ames Salmonella/microsome test and the containment system. Of the ten, only 1,2-dibromoethane gives positive results in the usual test procedure whereas seven of the ten are positive in the closed system. The specificity observed for the reversion of the tester strains and the lack of any significant effect of added rat liver "S-9" fractions suggest that halogenated alkanes are direct acting "base pair" type mutagens. The seven active compounds, listed in order of decreasing
mutagenicity for strain TA-100, are as follows: 1,2-dibromoethane; bromoethane; iodoethane; 1-bromopropane; 1-bromobutane; dichloromethane and 1,2-dichloroethane. 1,1,2-Trichloroethane, 1-chlorobutane and tetrachloromethane were inactive.

P-40
USE OF THE SPIRAL PLATER™ WITH THE SALMONELLA TEST FOR CHEMICAL MUTAGENS.
N.L. Gouse* and J.W. King, Department of Biological Sciences, University of Denver, and Chemical Division, Denver Research Institute, Denver, CO 80208.

An attempt was made to validate the spiral plating technique for use with the Salmonella assay for chemical mutagens. The Spiral Plater™ deposits a known volume of liquid on an agar surface in the form of an Archimedes spiral beginning at the center of a petri plate. The amount deposited decreases with increasing distance from the center. Chemicals such as MNNG were deposited directly on the bottom agar and overlaid with soft agar containing susceptible cells. After incubation, a clear zone was observed at toxic concentrations of the chemical, surrounded by a zone of revertants at mutagenic concentrations. In 15 cm plates, this zone was surrounded by an area of infrequent spontaneous revertants. Two counting templates were developed for 10 cm diameter plates. Dose-response curves for three different MNNG concentrations and two different cell lines (TA 1535, TA 100) were generated. The standard deviation using this method was equal to or less than that obtained using standard plating methods. Preliminary results indicate that dose-response curves for chemicals requiring activation can be obtained by spirally plating a mixture of the S9 plus mutagen. The use of the spiral plating technique provides results which are comparable to those obtained using standard methods. Because an entire dose-response curve is observed in one petri plate, this method is more economical than standard methods.

P-41
"A PORTABLE FIELD UNIT FOR IN SITU USE OF THE AMES TEST,"
P.R. Politte, M.E. Schrod, W.R. Lower; University of Missouri, Columbia, Missouri 65201

A portable apparatus has been developed which enables the Ames' Salmonella typhimurium test to be used in field research. Six strains (TA98, TA100, 1535, 1537, 1538, 1978) are used to survey ambient air for mutagenicity. The Ames' strains and overlay, minus the S9 fraction, are used. The plates, with controls, are placed in the unit and environmental air is drawn over the plates by means of vacuum pumps at the rear of the unit. After sampling, the plates may be returned to the laboratory for examination. The test has been run successfully, both in the lab and in the field. Surveys made near petrochemical complexes are statistically significant (at the 95% level) compared to residential or pristine areas. Like the original Ames' test, this modification is inexpensive, quick and effective. Many tests have been run in a single day, at different sites. In the future, this test may be used indoors to detect possible health hazards in the work setting.
MUTAGENICITY ASSESSMENT OF LIQUID EFFLUENTS FROM OIL SHALE TECHNOLOGIES
B. J. Barnhart, S. H. Cox, W. D. Spall, and R. T. Okinaka, Life Sciences Division, University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545

Chemicals and complex mixtures exhibiting mutagenic activity are considered to be potential hazards to people who come in contact with them. One strategy for determining whether or not chemicals are mutagens proposes the use of a tier approach in which the first tier is composed of short term in vitro bacterial and eucaryotic test systems. We are using the Ames Salmonella histidine-reversion test and the CHO/HGPRT mammalian cell tissue culture assay, both incorporating the rat liver microsome activation system as a first tier approach to assaying the relative mutagenicities of liquid effluents from various oil shale technologies. The product oil and a sludge oil from one horizontal modified in situ retort process gave strong positive results in the Ames test, 5.7 and 1.4 rev/µg total organic carbon (TOC), respectively. This product oil was, however, considerably less mutagenic than a surface retorted oil (26 rev/µg TOC). No mutagenic activity was observed for boiler blow-down, heater-treater and product waters, or for pilot plant light oil from the modified in situ process. In contrast to the shale oils, a Louisiana crude petroleum was non-mutagenic. Ames tests on various waters from each retorting process showed that water which separated in a holding tank from the surface retorted oil was the most mutagenic. This water also gave positive results in the mammalian cell mutagenesis assay. Other aqueous effluents are being tested in the mammalian cell assay. It is not known whether the large differences in activities between surface and modified in situ processed shale oils and waters is process- or site-specific. [This work was performed under the auspices of the United States Department of Energy.]

A COMPARISON OF THE CAPACITY OF FROG AND RAT LIVER HOMOGENATES TO ACTIVATE PROMUTAGENS IN THE Ames TEST. Albert M. Cheh, Alan B. Hooper, Craig Henke, Jill Skochdopole, and Robert G. McKinnell (intr. by Dante J. Picciano), University of Minnesota, Gray Freshwater Biological Institute, Navarre, MN 55392, and Department of Genetics and Cell Biology, St. Paul, MN 55108.

A mutagenic assay employing the frog, Rana pipiens, is currently under development (McKinnell, Picciano, and Schaad, Environmental Mutagenesis, in press). The Ames assay offers a simple means of comparing the metabolism of mutagens by different animal species. The Ames response obtained with frog liver S-9 was compared to the response with rat liver S-9, using the following compounds: benzo(a)pyrene, 2-aminofluorene, azobenzene, Sudan II, hydrazine, dibutylnitrosamine, diallate, tris-(2,3-dibromopropyl)phosphate, cyclophosphamide, quercetin, emodin, aflatoxin B1, quinoline, 1,2-dichloroethane, 7,12-dimethylbenzanthracene, and safrole. Of these compounds, all except quinoline, Sudan II, safrole, and 1,2-dichloroethane gave positive Ames responses with frog S-9. In general, the responses with frog S-9 were quantitatively lower than those obtained with Arochlor induced rat S-9; however, we have not yet determined the optimum induction procedure with frog liver. Safrole was negative with rat S-9, while the response to dichloroethane is very sensitive to the amount of activating enzyme present and might be positive with optimally induced frog S-9. Thus, only two of the 15 compounds positive with rat S-9 were definitely missed when tested with frog S-9. We feel that the frog assay appears to be promising from the standpoint of false negatives. Supported in part by NIH Grant 5RO1-ES 01605-02.
PREDICTING LONG TERM EFFECTS FROM SHORT-TERM TESTS DURING DRUG DEVELOPMENT
R. S. Lake and F. A. de la Iglesia*, Department of Toxicology, Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan

Several core batteries, grids and multiple decision point tier schemes have been proposed for deployment of mutagen/carcinogen screening tests. The pharmaceutical environment involves testing of a small number of high priority chemicals with highly defined pharmacological properties and thus "screening" tests per se are not adequate to define drug safety. A test scheme is proposed which integrates preclinical animal toxicity studies with short-term tests. A selection step, based on structure and pharmacological properties, precedes a 2 chamber group of tests. A flexible set of bacterial and mammalian cell mutagenicity assays to detect genotoxic agents is conducted in Chamber I along with preliminary toxicology. Compounds of acceptably low mutagenic and low general toxic potentials are then tested in Chamber II for subacute effects, reproduction-teratology, cell transformation and bioassays for the prediction of neoplastic potential in target organs. A second risk assessment precedes the recommendation for clinical trials. These short-term tests are chosen to provide maximum sensitivity as well as selectivity in covering genetic and epigenetic end points and include comprehensive in vitro microsomal systems to couple metabolic activation. This double-decision system may prove useful since drugs, unlike general chemicals, have a definite benefit/risk component influencing the overall assessment process. Thus, some drugs effecting bacterial point mutations by weak DNA structural damage mechanisms and without correlated positive effects in mammalian cell assays may be recommended for further development. Unequivocal positive results in either test chamber modify the critical path for development while unequivocal negative results would predict a negative outcome in long-term rodent bioassays.


Annually, millions of tons of benzidine derived azo dyes are produced and used in the manufacturing of paper products, textiles, leather goods and plastics. Epidemiological evidence suggests that occupationally exposed workers have a high risk of developing urinary bladder cancer. The carcinogenic potential of these dyes is believed to be due to the metabolic conversion of the dyes to benzidine in the animal's system.

In this study, the Ames Salmonella/microsome assay was used to evaluate the mutagenic potential of some proposed metabolites of the dyes Direct Black 38 and Pigment Yellow 12. Direct Black 38 and five of its metabolites, including benzidine and 4-aminobiphenyl, and three metabolites of Pigment Yellow 12 were tested at concentrations as high as 100 µg/plate using strains TA-1538, TA-98 or TA-100 with and without a mouse liver S-9 fraction. None of the metabolites of Black 38 were mutagenic without S-9 but all produced significant increases in revertants above background when S-9 was present. One metabolite of Yellow 12 was mutagenic without S-9 using TA-98 and the other two were active when the S-9 was added.

When urine from male Syrian hamsters treated with 100 mg/kg of Direct Black 38 was assayed in a modified test, mutagens were detected when an S-9 fraction was added. The major metabolite in the urine was found to be monoaceetylbenzidine by EC-GC assay. These results indicate that these widely used benzidine derived compounds may be metabolized by mammalian enzymes to form active mutagens.
COMPARATIVE MUTAGENIC ACTIVITY OF PARTICLE BOUND ORGANICS FROM COMBUSTION SOURCES. Joellen L. Huisingh and Larry Claxton, U. S. Environmental Protection Agency, Research Triangle Park, NC 27711. Respirable particles from a variety of combustion sources have the potential of being carcinogenic and mutagenic. The objective of these studies was to determine the relative biological activity of the organic material adsorbed on these particles in both in vitro and in vivo mutagenesis and carcinogenesis bioassays. The mobile combustion sources included three Diesel cars, a heavy-duty Diesel engine and a gasoline-catalyst car. The combustion sources selected for comparison included cigarettes, a coke oven, and a roofing tar pot. The organic extracts from these particle emissions were evaluated in a battery of mutagenesis and carcinogenesis bioassays. The mutagenesis assays included gene mutation and DNA damage assays in both microbial and mammalian cells. Each extract was tested in the Salmonella typhimurium/microsome plate incorporation test. The mutagens present in the Diesel samples were primarily direct acting, frameshift mutagens. The other combustion sources also exhibited mutagenic activity typical of frameshift mutagens either requiring or significantly enhanced by the addition of a metabolic activation system. The assay was performed at a minimum of five doses with and without microsomal activation in five tester strains. The specific mutagenic activity ranged over two orders of magnitude for the Diesel samples. Although the most active sample was a Diesel vehicle emission, the comparative sources also showed a range of activities comparable to the range seen with Diesel vehicles.

MUTAGENIC AND LEthal EFFECTS OF A SERIES OF m-DIAminOBENzE DERIVATIVES IN SALMONELLA TYPHIMURIUM AND SACCHAROMYCES CEREVISIAE. M. M. Shahin, A. Bugaut* and C. Kalopissis*, L'Oréal Research Laboratories, L'Oréal, 1, Avenue de St. Germain, 93601 Aulnay-sous-Bois, France.

In our structure activity relationship studies, we investigated the mutagenicity of m-diaminobenzenes (m-phenylenediamine) and four 2,4-diaminoalkylbenzenes (methyl, ethyl, isopropyl or n-butyl-group) in Salmonella typhimurium strains TA100, TA1538 and TA98 as well as in Saccharomyces cerevisiae strain XV185-14C in the absence and presence of Aroclor 1254 induced S-9. In Salmonella typhimurium strains m-diaminobenzene was found to be the most active mutagen, followed by 2,4-diaminotoluene and 2,4-diaminooctylbenzene, respectively. Negative response was observed for both 2,4-diaminoisopropylbenzene and 2,4-diamino-n-butylbenzene. Thus, depending on the size of the substituting alkyl group at C1 position of 2,4-diaminoalkylbenzene, a decline and loss of mutagenic activity was observed. In Saccharomyces cerevisiae strain XV185-14C, for the induction of gene mutations, all five compounds were found to be inactive. However, a correlation has been observed between the lethal effect and the chemical structure of the tested compounds. The larger the size of the substituent at C1 position of 2,4-diaminoalkylbenzene, the more toxic the compound was found to be.

FORMATION OF PURE AND MOSAIC MUTANT CLONES AND THEIR RELEVANCE IN CARCINOGEnESIS. M. A. Hannan* and A. Masim†. †Ephraim McDowell Community Cancer Network, 915 S. Limestone, Lexington, KY 40536 and 2Div. of Biological Science, NRC of Canada, Ottawa, K1A 0R6.

Studies were undertaken to elucidate the mechanisms underlying the formation of pure and mosaic mutant clones by different mutagens. For this study, a homogeneous population of C1 cells obtained from the haploid Saccharomyces cerevisiae strain, Cl6-11C were used. The cells were treated
with different physical and chemical mutagens to obtain both high and low levels of survival. Cells were plated on YEPD agar and colonies showing red pigmentation (a characteristic of the adenine requiring mutants) were scored. The frequency of 'whole red' and 'sectored' (red/white) colonies was estimated for each of the mutagens at a comparable and high survival level. It was observed that, at 60-90% survival levels, some mutagens like UV and EMS produce mainly pure mutant clones while others like nitrous acid and EMS produce mainly mosaics. These data are interpreted to mean that the former mutagens produce mutations before DNA replication while the latter class may do so via a replication-dependent process. The results will be discussed to demonstrate the significance of replication-dependent and replication-independent mutagenesis in initiation of carcinogenesis. The usefulness of studies on pure and mosaic mutations will be emphasized particularly in relation to the characterization of environmental carcinogens that would require cell proliferation for tumor initiation.

**P-49**


A modified protocol has been developed for the yeast mitotic recombination assay employing *Saccharomyces cerevisiae* D3 for the detection of environmental genotoxic/mutagenic chemicals. The yeast *S. cerevisiae* D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound is determined from the number of red-pigmented colonies appearing on the plates. Instead of exposing $10^6$ D3 organisms to the test chemical in suspension with subsequent diluting and plating for survivors and mitotic recombinants, about $10^5$ D3 organisms are preincubated with the test chemical, with and without metabolic activation, for 2-4 hrs at 30°C. Two ml of top agar is then added and the mixture is poured onto the plate selective for adenine homozygosity. The plates are then incubated for 2 days at 30°C followed by 2 days at 4°C to enhance the development of the red pigment. The advantages of this system are reduction in time, cost, data transformation, and in the amount of chemical needed. With this method, a positive response is indicated by a reproducible dose-related increase in the number of mitotic recombinants per plate. Plating for survival is not performed as testing up to the toxic or maximum soluble level is done. In this new system, we have tested 1,2,3,4-diepoxybutane, β-propiolactone, 4-nitroquinoline-N-oxide, various pesticides, and some chemicals previously reported as weak mutagens in the D3 assay, such as safrole.

**P-50**

A SIMPLE, RAPID PLATE ASSAY FOR MITOTIC RECOMBINATION. B.A. Kunz, B.J. Barclay and R.H. Haynes, Department of Biology, York University, Toronto, Ontario, Canada, M3J 1P3.

It is important to test the recombinagenicity of environmental chemicals for the following reasons: 1) certain chemicals are recombinagenic but not mutagenic 2) induced recombination might cause expression of recessive tumorigenic mutations or of recessive alleles detrimental to fetal development. We have developed a simple assay for detecting mitotic recombination in *S. cerevisiae*. The diploid strain D7 is heterozygous for cyh2, a recessive defect that confers cycloheximide resistance. Both gene
conversion and crossing over can result in homozygosity for cyh2 and so allow growth on cycloheximide medium. To perform the assay, cells of D7 are spread on nutrient agar and the chemical to be tested is added to a center well in the plate. The cells grow while being exposed to a gradient of chemical concentration formed as the agent diffuses into the medium. If the chemical is recombinagenic then following replica-plating on to cycloheximide medium a ring of resistant colonies will emerge during incubation. The contribution of monosomy to cycloheximide resistance can be determined by using other markers located on the same chromosome arm as cyh2. To increase the sensitivity of the assay we have constructed a D7 variant (W73) homozygous for a defect in excision repair (rad3-2). A number of compounds including EMS, 5dUMP, nitrosoguanidine, methotrexate and various sulfonamides are positive in this test. However some of these chemicals are not mutagenic at the nuclear level. Thus we consider it essential to include an assay for mitotic recombination in any proposed battery of tests for genetic activity. (Supported by NSERC Canada)

P-51

We have previously reported a plant-microbe bioassay with which we can test chemicals for mutagenic activity under conditions normally encountered in the environment. Our approach utilizes in vivo and in situ methodologies. The in vivo method involves exposing Zea mays plants to a chemical, homogenizing the plant tissues, and making an extract. Microbial indicator organisms are then exposed to the extracts and appropriate genetic events scored. The in situ approach utilizes the waxy (wx) locus of Z. mays microgametophytes (pollen grains). Homoaallelic wx-C/wx-C plants grown in the presence of the chemical are monitored for Wx revertants. Using these two approaches we have detected genetic activity in the insecticides Chlor dane, Heptachlor, and Dyfonate, and in the herbicides Cycle, Cyanazine, Simazine, and Propachlor. With the exception of Dyfonate, none of these agents are mutagenic directly or following mammalian S9 metabolism. We are also developing an in vitro complement to our in vivo and in situ assays. The in vitro method involves incubating a test chemical with an untreated plant tissue homogenate supplemented with cofactors. This method has the advantage of being similar to the standard mammalian activation procedures. Using the in vitro assay we have been able to activate aflatoxin B1 to a mutagenic form, but have been unable to deactivate N-methyl-N'-nitro-N-nitrosoguanidine, a direct acting chemical readily deactivated in vitro by mammalian tissues. These data suggest that plants have an ability to activate promutagens, and that some differences exist between plant and animal activation systems. This research supported in part by USEPA Contract 68-02-2704 and by NSF-URP SPI-7683612.

P-52
CYTOGENETIC TOXICOLOGIC TESTING WITH DOGS. R. Daniel Benz and Patricia A. Beltz, University of California, Irvine - Toxic Hazards Research Unit, P. O. Box 3067, Overlook Branch, Dayton, Ohio 45431

Dogs are large animals as are humans and it is very simple to obtain blood samples from dogs without harming the animals. Because of this we have used purebred beagles for cyrogenetic toxicologic testing as a model to form a firm validation basis in preparation for studies in which humans are tested for the presence of induced cyrogenetic damage. We have done a series of experiments to test the ability of several classes of chemicals to induce cyrogenetic damage in the forms of sister chromatid exchanges (SCEs) and micronuclei in canine peripheral lymphocytes. Cells
were exposed directly in vitro and live animals were exposed by inhalation and blood samples taken periodically during the exposure. Chemicals tested were mitomycin C (MMC), ethylmethanesulfonate (EMS), methylmethanesulfonate (MMS), acetone, ethanol, methanol, dimethylsulfoxide; hydrazine, monomethylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, dimethylaminonitrosamine; jet fuels JP-4, -5, -10, decalin, methylcyclohexane; and marine diesel fuel. Not all chemicals were tested for both SCEs and micronuclei nor were all tested both in vitro and in vivo. In the tests that were done, none of these chemicals were found to induce SCEs or micronuclei except, as expected, MMC, EMS and MMS, and also very high doses of ethanol. The results of the short term cytogenetic toxicologic tests done using live animals were compared to classic toxicologic tests done using the same animals. Not surprisingly, the short term tests are not good predictors of short term toxic effects (e.g. changes in body weight). Comparisons of the results of the cytogenetic tests with long term effects such as oncogenesis have not been made because the animals involved are still being held for future observation.

P-53
A METHOD FOR DETECTING REACTIVE ELECTROPHILES IN ENVIRONMENTAL SAMPLES.
Albert M. Cheh and Robert E. Carlson, Gray Freshwater Biological Institute, University of Minnesota, Navarre, MN 55392.

Most ultimate mutagens are electrophiles. The nucleophile 4-nitrothiophenol (NTP) reacts readily with electrophiles to form thioethers which have an absorption maximum at ca. 350 nm. We have developed a procedure for the detection of reactive electrophiles in aqueous solution by combining NTP reaction with high-performance liquid chromatographic (HPLC) separation of the thioether products. This procedure has been demonstrated using alkyl halides and epoxides at sub-ppm concentrations. NTP should be a useful reagent for studying environmental samples because:
(1) Those compounds which react covalently with NTP are specifically electrophiles and potentially mutagens.
(2) The electrophiles will be labelled with a 350 nm chromophore, which will allow the quantitation of trace (ppb) levels of electrophiles after preconcentration, even in the presence of ppm quantities of total organics which absorb mainly below 300 nm.
(3) The kinetics of reaction (reactivity) of the electrophiles can be determined readily by HPLC analysis.

P-54
COVALENT BINDING OF CHEMICAL CARCINOGENS AND MUTAGENS TO RAT HEMOGLOBIN.
M.A. Pereira and L. Chang, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Cincinnati, OH 45268.

The alkylation of hemoglobin is a proposed dose monitor for environmental chemical carcinogens and mutagens. The binding of direct acting alkylating mutagens to hemoglobin has been determined. The determination of the binding of the various chemical classes of indirect acting mutagens was needed prior to the initiation of a research program designed to monitor human environmental exposure to chemical mutagens by the detection of their covalent binding to hemoglobin.

Male rats were administered orally carbon-14 radiolabeled chemicals. Twenty-four hours later blood was obtained by cardiac puncture and the red blood cell lysate containing the hemoglobin separated. The heme was removed by precipitation of the globin with 1% HCl-acetone. The binding of fifteen direct and indirect acting carcinogens and mutagens to rat hemoglobin was determined. The binding to hemoglobin was dose dependent and the efficiency of binding to hemoglobin ranged from 0.07 to 2.3% of
an oral dose. The order of binding to hemoglobin ranked in decreasing efficiency was Methyl Methanesulphonate, DimethylNitrosamine, N-Nitroso-methyleurea, N-Nitroso-N-Ethyleurea, Benzo(a)pyrene, Methyl-N'-Nitro-N-Nitrosoquinidine, 3-Methycholanthrene, Nitrosodinethamine, Benzene, 2-Acetylaminofluorene, 7,12-Dimethylbenz(a)anthracene, Aflatoxin B1, Benzidine, Chlороform, and Carbon Tetrachloride. The binding of chemical carcinogens and mutagens to hemoglobin would appear to be generic so that it could be developed into a dose monitor for the majority of known chemical carcinogens and mutagens.

P-55
HUMAN 3-METHYLDENENINE-DNA GLYCOSYLASE AS A PROBE FOR DETERMINING ALKYLATION DAMAGE AND REPAIR IN HUMAN CELLS. Thomas P. Brent, intr. by A.D. Welch, St. Jude Children's Research Hospital, Memphis, TN 38101.

3-Methyldenene-DNA glycosylase was partially purified from human lymphoblasts and used as an enzymatic probe to assay the amounts of 3-methyladenine in DNA from cultured human fibroblasts after treatment with dimethyl sulfate. Aside from this specific alkylation product, the total number of alkylated bases was estimated after depurination by heating at neutral pH. Both enzyme-induced and heat-induced apurinic sites were converted to strand-breaks and estimated after alkaline sucrose-gradient sedimentation. The results indicate that 3-methyladenine in cultured human fibroblasts is rapidly excised, with a half-life of about 2 hours. The rest of the alkylated purines (mostly 7-methylguanine) are removed more slowly with a half-life of about 20 hours; however, this is much faster than expected for spontaneous hydrolysis and suggests that 7-methylguanine also is excised by an active enzymatic process. 3-Methyldenene-DNA glycosylase is further being used as a probe to determine if the capacity for excision of 3-methyladenine by normal human cells becomes saturated at high levels of alkylation, and if such capacity is reduced in human mutants that show elevated sensitivity to alkylating agents. The analytical procedure used also can identify potential defects in steps of the excision repair pathway subsequent to the glycosylase, i.e., apurinic endonuclease through DNA ligase. (Supported by NIH Grant CA14799 and by ALSAC).

P-56
NORHARMAN & ELLIPTICINE: A COMPARISON OF THEIR ABILITIES TO INTERACT WITH DNA IN VITRO.
J. Ashby, B. Elliott and J. A. Styles, Imperial Chemical Industries, Ltd., Alderley Park Nr. Macclesfield, Cheshire UK 5K10 4TJ

P-57
DEVELOPMENT AND APPLICATIONS OF AN EQUILIBRIUM DOSE RESPONSE MODEL
D.J. Schaeffer Illinois EPA, Springfield, IL., 62706 K.G. Janardan, Sangamon State University, Springfield, IL., 62708, H.W. Kerster, California State University, Sacramento CA, 95819

The Lagrangian Poisson Distribution (LPD), which was developed as a model for explaining the induced production of chromosome aberrations, has been shown to provide information on the free energy of aberration production, threshold level, and low-dose response (Janardan et al. Bioscience 29, 599, 1979). Thus, g2, the parameter of the LPD, is the chemical equilibrium constant at equilibrium. Using this as a starting point, the mass action relationship between g2 and dose was developed by considering the initial dose to be randomly "filtered" prior to reaction. This affords a new does-response relationship which is a multiplicative power function of the dose. The inflection
point of the dose-response curve is interpreted as the threshold under certain conditions. For radiation data, the thresholds estimated from over 40 data sets are in the range 10-60 rads, for experiments employing doses from 0-5000 rads. Comparison of this model in the low dose region with commonly employed models will be made using data from radiation and chemically induced damage or death.

P-58

GENETIC SEGREGATION IN MICE DETECTED BY TWO-DIMENSIONAL ELECTROPHORESIS.

A.S. RaJ and R.R. Marshall#, Biology Department, York University, Downsview (Toronto), Canada

Liver proteins from mice were separated by two-dimensional electrophoresis (O'Farrel, P.H., J. Biol. Chem. 250: 4007-4021, 1975). This technique combines isoelectric focusing with SDS polyacrylamide gel electrophoresis and separates proteins according to pI in the first direction and molecular weight in the second. We have tested liver samples from C57BL/6J females and C3H male inbred strains, F1 hybrids, and F2 progeny. Each parent strain showed a characteristic polypeptide spot pattern that differed in several ways (both qualitative and quantitative) from the other. In the F1 hybrids all spots present in either parent could be detected but segregation was observed in the F2. Using the "silver staining" technique (Merril et al., Proc. Natl. Acad. Sci. USA., 76:4335-4339, 1979) we have resolved about 930 spots on a single gel. This indicates that there is at least a potential for detection of induced mutations at many different loci for which these preliminary results form a basis.

P-59


Primordial oocytes in the juvenile mouse are extremely vulnerable to destruction by both ionizing radiation and certain chemicals such as polycyclic aromatic hydrocarbons (PAHs). We have used this sensitive, whole-animal system for cytotoxicity determinations on a wide range of chemicals. The 45 agents tested so far give a 4-decade range of results. Mutagenic PAHs, direct acting alkylating agents, and a number of other mutagen-carcinogens are strongly positive; other agents are less so; while aromatic amines, dialkylamines, chlorinated hydrocarbons, and metals have so far been negative. No non-mutagen-carcinogen has yet been found to be positive. Technical improvements in procedure have increased the assay's practicality, facilitating 5-point dose-response determinations, with 5 animals per point, completed within three weeks. Ovaries are histologically sectioned in groups, 5 per paraffin block, to minimize processing effort. Surviving primordial oocytes are then microscopically enumerated in each ovary by counting in every 20th section. This assay is highly sensitive: ED50s approach those of both short-term bacterial mutagenicity tests and long-term animal carcinogenicity studies. The assay yields quick results, yet uses the whole mammal, with transport, metabolism, and enzymatic activation intact.

Work performed under the auspices of the U.S. DOE under contract number W-7405-ENG-48 and U.S. EPA interagency agreement EPA-IAG-05-E681-AQ.
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<td>Registration</td>
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<tr>
<td>8:45 a.m.</td>
<td>Mulligan Testing</td>
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<tr>
<td>9:00 a.m.</td>
<td>Cumberland Foyer</td>
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<tr>
<td>9:15 a.m.</td>
<td>Jefferson/Franklin Room</td>
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<tr>
<td>9:30 a.m.</td>
<td>Shelby Room</td>
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<tr>
<td>9:45 a.m.</td>
<td>Woodland Room</td>
</tr>
<tr>
<td>10:00 a.m.</td>
<td>2:00 P.M. Annual Business Meeting and Awards</td>
</tr>
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</tr>
<tr>
<td>11:00 a.m.</td>
<td>Woodland Room</td>
</tr>
<tr>
<td>11:15 a.m.</td>
<td>Jackson Room</td>
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There are no designated times for paper presentations. The papers will be displayed for a 15-minute period and questions will be answered. The poster session will indicate when the author will be present for discussion and questions.