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Report # 7

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TSCA 8(e) COMPLIANCE AUDIT PROGRAM: The attached information represents a submission under the TSCA Section 8(e) Compliance Audit Program and Cap Agreement cited below.

CAP Agreement Identification Number: 8ECAP-0056



Company: Minnesota Mining and Manufacturing (3M)
Corporate Product Responsibility
3M Center, Bldg. 225-3N-02
St Paul, Minnesota 55144-1000

Contact: Georjean L Adams 7/27/92
Georjean L Adams, Manager Regulatory Affairs
612/737-4795

Chemical Identity:
bis 5,5'(N-ethyl-benzo[~~a~~]carbazoyl) phenol methane
CAS#82926-35-0

Title of Study: "Mutagenicity Evaluation of T-2380 Du in the Sister Chromatid Exchange Assay with Chinese Hamster Ovary (CHO) Cells Final Report"

"*in vitro* Microbiological Mutagenicity Assays of 3M Company Compounds T-2379 Du and T-2380 Du"

Summary of Adverse Effects:
Positive *in vitro* mutagenicity assays

Comments: T-2380 Du is a sample of the subject chemical
T-2379 Du is a different chemical and was not positive

Confidential: X No

* * * * *
3M does not believe this product presents unreasonable risks to health and the environment as used by 3M and its customers.

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GENETICS ASSAY NO. 4226

LBI SAFETY NO. 3929

MUTAGENICITY EVALUATION OF

T-2380 DU

IN THE
SISTER CHROMATID EXCHANGE ASSAY
WITH CHINESE HAMSTER OVARY (CHO) CELLS

FINAL REPORT

SUBMITTED TO:

3M COMPANY
3M CENTER
ST. PAUL, MINN. 55101

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20990

REPORT DATE: JULY 1979

 **BIONETICS**
Litton

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PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-VIII. Items I-IV provide sponsor and compound identification information, type of assay, and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item VI identifies the supervisory personnel. Item VII identifies the tables and/or figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report, entitled STUDY DESIGN, describes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices.

All test and control results presented in this report are supported by raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795.

Copies of the raw data will be supplied to the sponsor upon request.



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- I. SPONSOR: 3M Company
- II. MATERIAL TESTED
 - A. Client's Identification: T-2380-DU
 - B. Genetics Assay No.: 4226
 - C. Date Received: April 24, 1979
 - D. Physical Description: White powder
- III. TYPE OF ASSAY: Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells (CHO)
- IV. PROTOCOL NO.: 438
- V. STUDY DATES:
 - A. Initiation Date: May 22, 1979
 - B. Completion Date: June 12, 1979
- VI. SUPERVISORY PERSONNEL
 - A. Study Director: Daniel Stetka, Ph.D.
 - B. Laboratory Supervisor: Helen Lebowitz
- VII. RESULTS
- VIII. INTERPRETATION OF RESULTS:

The results of this assay are presented in Tables 1 and 2.

The test compound, T-2380-DU, is evaluated here in terms of its ability to induce sister chromatid exchanges (SCEs) in CHO cell's when used directly and also in the presence of a metabolic activation system that contains liver microsomal enzymes from Aroclor-induced rats.

Prior to dosing of the test cultures, a stock solution of T-2380-DU was prepared in DMSO at 100 mg/ml. Serial dilutions were then performed using the same solvent so that final concentrations were achieved with the addition of 0.1 ml of solution per culture (i.e., per 10 ml of medium).



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VIII. INTERPRETATION OF RESULTS (Continued)

Results of the direct (nonactivation) assay are presented in Table 1. Doses of T-2380-DU covered 5 orders of magnitude, and all but the lowest dose induced significant increases in SCE frequency. Although the frequencies are not exceptionally high (2 x solvent control value at the highest dose), they do tend to increase with dose. Thus, it must be concluded that the test compound, at least when used with the present solvent (DMSO), does induce small but statistically significant increases in SCE frequency at doses as low as 0.10 µg/ml.

With activation the results were essentially identical (Table 2). Solvent control frequency was unusually high, so only the 3 highest dose levels of the test compound induced significant increases in SCE frequency; but frequencies did tend to increase with dose, and they were similar to those observed in the nonactivation series. The positive control compound, DMN, induced a significant increase in SCE frequency, indicating that the activation system was functional. Thus, the nearly identical responses observed with and without activation suggest that the liver microsomal enzymes had no effect on the SCE-inducing capacity of this compound.

IX. CONCLUSIONS

In conclusion, T-2380-DU is a weak inducer of SCE, with and without activation, under the conditions of this assay.

Submitted by:

Study Director



Daniel Stetka, Ph.D.
Section Leader
Animal Genetics and
Cytogenetics
Department of Genetics
and Cell Biology

Date

Reviewed by:



David J. Brusick, Ph.D.
Director
Department of Genetics
and Cell Biology

7/17/79
Date



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TABLE 1
SCE FREQUENCIES IN CELLS EXPOSED TO
T-2380-DU

| Treatment | Dose | No. of Chromosomes | No. of SCE's | Nonactivation | |
|----------------------------|--------------------|--------------------|--------------|-------------------------|----------|
| | | | | SCE/Chromosome \pm SE | SCE/Cell |
| Neg. Control (Medium) | -- | 360 | 211 | 0.586 \pm .040 | 11.72 |
| Solvent Control DMSO | -- | 347 | 262 | 0.755 \pm .047 | 15.10 |
| Positive Control EMS | 0.5 μ i/ml | 372 | 1197 | 3.218 \pm .093 | 64.35** |
| Test Compound T-2380-DU | 0.01 μ g/ml | 360 | 280 | 0.778 \pm .046 | 15.56 |
| T-2380-DU | 0.10 μ g/ml | 341 | 360 | 1.056 \pm .056 | 21.11** |
| T-2380-DU | 1.00 μ g/ml | 350 | 344 | 0.983 \pm .053 | 19.66** |
| T-2380-DU | 10.00 μ g/ml | 347 | 387 | 1.115 \pm .057 | 22.31** |
| T-2380-DU | 100.00 μ g/ml | 352 | 430 | 1.222 \pm .059 | 24.43** |
| T-2380-DU | 1000.00 μ g/ml | 333 | 470 | 1.411 \pm .065 | 28.23** |

**Significantly greater than solvent control value, $P < 0.01$ (Student's t-test)



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TABLE 2
SCE FREQUENCIES IN CELLS EXPOSED TO
T-2380-DU

| Treatment | Dose | No. of Chromosomes | No. of SCE's | Activation | |
|---------------------------|--------------------|--------------------|--------------|-------------------------|----------|
| | | | | SCE/Chromosome \pm SE | SCE/Cell |
| Negative Control (Medium) | -- | 359 | 256 | 0.713 \pm .045 | 14.26 |
| Solvent Control DMSO | -- | 359 | 356 | 0.992 \pm .053 | 19.83 |
| Positive Control DMN | 0.3 μ l/ml | 372 | 487 | 1.309 \pm .059 | 26.18** |
| Test Compound T-2380-DU | 0.01 μ g/ml | 350 | 303 | 0.866 \pm .050 | 17.31 |
| T-2380-DU | 0.10 μ g/ml | 348 | 350 | 1.006 \pm .054 | 20.11 |
| T-2380-DU | 1.00 μ g/ml | 347 | 358 | 1.032 \pm .055 | 20.63 |
| T-2380-DU | 10.00 μ g/ml | 341 | 463 | 1.358 \pm .063 | 27.16** |
| T-2380-DU | 100.00 μ g/ml | 359 | 503 | 1.401 \pm .062 | 28.02** |
| T-2380-DU | 1000.00 μ g/ml | 354 | 455 | 1.285 \pm .060 | 25.71** |

**Significantly greater than solvent control value, $P < 0.01$ (Student's t-test).



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PROTOCOL

1. OBJECTIVE

The objective of this in vitro assay is to evaluate the ability of a test article to induce sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells, with and without metabolic activation.

2. MATERIALS

A. Indicator Cells

Cells to be used in this assay are obtained from the American Type Culture Collection Repository No. CCL61, Rockville, MD. The original cells were obtained from an ovarian biopsy of a Chinese hamster. This is a permanent cell line with an average cycle time of 10 to 12 hours.

B. Medium

CHO cells for this assay are grown in Ham's F12 medium supplemented with 10% fetal calf serum (FCS). The cells are split back to 3×10^5 per 75-cm² plastic flask and fed 24 hours prior to treatment with 10 ml of fresh medium.

C. Control Articles

1. Negative control article

The solvent for the test article will be used as the solvent or vehicle control article.

2. Positive control articles

Ethylmethane sulfonate (EMS), a chromosome breaking agent that induces SCE, will be dissolved in culture medium and used as a positive control article for the nonactivation studies at a final concentration of 0.5 μ liters/ml.

Dimethylnitrosamine (DMN), an SCE inducer that requires metabolic biotransformation by microsomal enzymes, will be used as a positive control article for activation studies at a final concentration of 0.3 μ liters/ml.



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SISTER CHROMATID EXCHANGE IN CHINESE HAMSTER OVARY CELLS

3. EXPERIMENTAL DESIGN

A. Toxicity and Dose Determination

The solubility, toxicity and doses for the test article may be determined prior to screening. The effect of each test article on the survival of the indicator cells will be determined by exposing the cells to a wide range of article concentrations in complete growth medium. Toxicity will be measured as the loss in growth potential of the cells induced by 4-hour exposure to the test article followed by a 24-hour expression period in growth medium. Doses will be selected from the range of concentrations by bracketing the highest dose that shows no loss in growth potential with at least one higher and three lower doses. Otherwise, either 1) the Sponsor may indicate the dose levels; or 2) a geometric series of doses will be employed, with the highest dose being perhaps limited by solubility, but in any case not to exceed 5 mg/ml. In the latter, the doses will cover at least four orders of magnitude, and all doses that yield sufficient numbers of scorable metaphase cells will be considered in the analysis.

B. Cell Treatment

1. Nonactivation assay

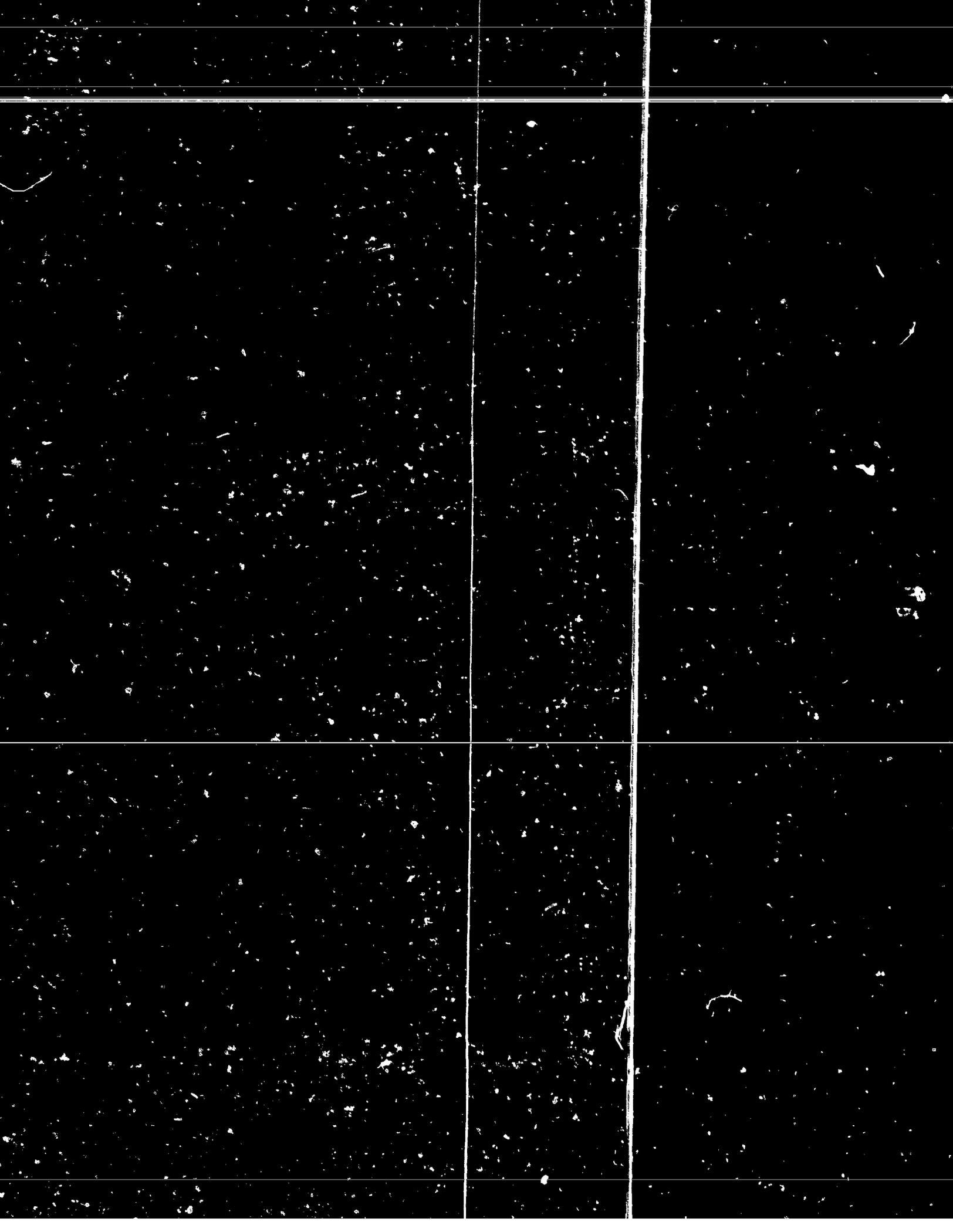
Approximately 10^6 cells will be treated in growth medium with the test article at predetermined doses, and then incubated at 37°C for 2 hours on a rocker. The exposure period will be terminated by washing the cells twice with saline. Then, 5-bromo-2'-deoxyuridine (BrdU; $20\ \mu\text{M}$ final concentration) will be added to the culture tubes and incubation continued in the dark for 24 to 30 hours. Longer times will often be necessary to permit cell passage through two DNA replication cycles in the presence of BrdU following treatments that cause mitotic delay. This will be determined experimentally. Colcemid will be added for the last 3 hours of incubation (final concentration $2 \times 10^{-7}\text{M}$), and metaphase cells will be collected by mitotic shake-off (Terasima and Tolmach, 1961). These cells will be swollen with 0.075M KCl hypotonic solution, then washed 3 times in fixative (methanol:acetic acid 3:1), dropped onto slides and air-dried.

2. Activation assay

The test article will be tested in the presence of an S9 rat liver activation system. This assay will differ from the non-activation assay in that S9 reaction mixture will be added to the growth medium, together with the test article, for 2 hours. The exposure period will be terminated by washing the cells twice with saline. From this point they will be treated as described above.



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SISTER CHROMATID EXCHANGE IN CHINESE HAMSTER OVARY CELLS

4. PREPARATION OF S9 REACTION MIXTURE

Fischer 344 male rats normally will be used as the source of hepatic microsomes. Induction with Aroclor 1254, or another agent specified by the Sponsor, will be performed by injections 5 days prior to kill. After decapitation and bleeding, the liver will be immediately dissected from the animal using aseptic technique and placed in ice-cold 0.25M sucrose buffered with Tris at pH 7.4. When an adequate number of livers has been obtained, the collection will be washed twice with fresh buffered sucrose and completely homogenized. The homogenate will be centrifuged for 10 minutes at 9,000 x g in a refrigerated centrifuge, and the supernatant (S9) from this centrifuged sample will be obtained and frozen at -80°C until used in the activation system. The fraction may be obtained from induced or noninduced rats or other species, as requested. This S9 fraction will be added to a "core" reaction mixture to form the activation system described below.

| Component | Final Concentration per Milliliter |
|---------------------|---------------------------------------|
| NADP (sodium salt) | 2.4 mg |
| Isocitric acid | 4.5 mg |
| Homogenate fraction | 15 μ liters |

5. STAINING AND SCORING OF SLIDES

Slides will be stained for 10 minutes with Hoechst 33258 (5 μ g/ml) in M/15 Sorensen's buffer (pH 6.8), mounted in the same buffer and exposed to ultraviolet (UV) light from a mercury lamp for the amount of time required for sister chromatid differentiation.* Following UV exposure the slides will be stained with 10% Giemsa for 10 minutes and then mounted in Depex.

Second division cells (M2 cells) will be scored for the frequency of SCE's per cell and per chromosome. The proportions of cells in the first, second and third divisions (i.e., M1, M2 and M3 cells) can also be determined, if desired. For SCE analysis, 20 M2 cells will typically be scored at each dose level.

For control of bias, all slides will be coded prior to scoring and scored blind.

*Modification of Perry and Wolff (1974) technique.

SISTER CHROMATID EXCHANGE IN CHINESE HAMSTER OVARY CELLS

6. EVALUATION CRITERIA

Data will generally be presented in table form. Interpretation will be based on the increase in SCE frequency as a function of dose and/or on the statistical significance of increases above the background or "spontaneous" level. The t-statistic will be calculated, and an SCE frequency increase will be considered positive if $p < 0.05$ and there is an indication of a positive dose response.

7. REFERENCES

Perry, and Wolff, S.: New dye technique for the differential staining of sister chromatids. *Nature*, 251:156-158, 1974.

Terasima, T. and Tolmach, L.J.: Changes in X-ray sensitivity of HeLa cells during the division cycle. *Nature*, 190:1210-1211, 1961.

8. RECORDS TO BE MAINTAINED

All raw data, protocol, modifications, test article weight and dispensation records and correspondence between LBI and the Sponsor will be maintained in a central file within the Department of Genetics and Cell Biology. These records will be filed under Departmental assay number and held up to 2 years following submission of the final report to the Sponsor. After 2 years they will be transferred to the LBI Archives for permanent storage.

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20990

LBI Assay No. 4226

TYPE OF STUDY Sister Chromatid Exchange in CHO cells assay

This final study report was reviewed by the LBI Quality Assurance Unit on 7/13/79. A report of findings was submitted to the Study Director and to Management on 7/16/79.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately every three months to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Mitchell S. Ehlis
Auditor, Quality Assurance Unit

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IN VITRO MICROBIOLOGICAL MUTAGENICITY
ASSAYS OF 3M COMPANY COMPOUNDS T-2379 D,
AND T-2380 D

Final Report

January 1979

By: Vincent F. Simon, Ph.D., Manager
Microbial Genetics Program
Nancy Marx, Microbiologist

Prepared for:

3M COMPANY
Medical Department
General Offices
3M Center
St. Paul, Minnesota 55101

Attention: Toxicology Services

SRI Project LSC-4442.-016

Approved:

David C. L. Jones

David C. L. Jones, Director
Toxicology Laboratory

W. A. Skinner

W. A. Skinner, Executive Director
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0015

SUMMARY

SRI International examined 3M Company compounds T-2379 Du and T-2380 Du for mutagenic activity with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium in the standard Ames Salmonella/microsome assay and with the yeast Saccharomyces cerevisiae D3. Each assay was performed in the presence and in the absence of a rat liver metabolic activation system. T-2379 Du was neither mutagenic nor recombinogenic in either assay. T-2380 Du was mutagenic in assays with strain TA100 with metabolic activation but was not recombinogenic in S. cerevisiae.

Note: 5,5'-(phenylmethylene)bis(11-ethyl-11H-benzo(a)carbazole) is T-2380

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WITHOUT 3M WRITTEN PERMISSION.

0016

INTRODUCTION

SRI International examined 3M Company compounds T-2379 Du and T-2380 Du for mutagenicity by in vitro microbiological assays with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium in the standard Ames Salmonella/microsome assay and with the yeast Saccharomyces cerevisiae D3. An Aroclor 1254-stimulated, rat liver homogenate metabolic activation system was included in the assay procedures to provide metabolic steps that the bacteria either are incapable of conducting or do not carry out under the assay conditions.

The assay procedure with S. typhimurium has proven to be 80 to 90% reliable in detecting carcinogens as mutagens, and it has about the same reliability in identifying chemicals that are not carcinogenic.¹ The assay procedure with S. cerevisiae is about 60% reliable in detecting carcinogens as agents that increase mitotic recombination.² However, because the assay systems do not always provide 100% correlation with carcinogenicity investigations in animals, neither a positive nor a negative response conclusively proves that a chemical is hazardous or nonhazardous to man.

METHODS

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal agar plates containing a trace of histidine, only those cells that revert to histidine independence (his⁺) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions in many cases, this growth is essential for mutagenesis to occur. The his⁺ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased 2- to 100-fold, usually in a dose-related manner.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.¹⁻⁵ In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa⁻) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (bio⁻) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB⁻). The rfa⁻ mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrB⁻ mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his⁺ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.⁵ In addition, plasmid pKM101 confers resistance to the

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antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)-pyrene, aflatoxin B₁, and 7,12-dimethylbenz(a) anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthracenes, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4° C in minimal agar plates, supplemented with a trace of biotin and an excess of histidine. The plates with the plasmid-carrying strains contain, in addition, ampicillin (25 µg/ml) to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every month from single colony isolates that have been checked for their genotype characteristics (his, rfa, uvrB, bio) and for the presence of plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amino type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.^{4,6-8} Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.⁶ In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of Aroclor 1254 (a mixture of polychlorinated biphenyl). This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum.

On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenated with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of MgCl₂ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 3.35 ml of H₂O.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.⁹ When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygous by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various recombinogenic agents. The recombinogenic activity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on test plates.¹⁰

A stock culture of S. cerevisiae is stored at 4° C. For each experiment, broth containing 0.05% MgSO₄, 0.15% KH₂PO₄, 0.45% (NH₄)₂SO₄, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 37° C with shaking.

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The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged and the cells are resuspended at a concentration of 10^6 cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.00 ml of the overnight broth culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of the test chemical
- 0.3 ml of buffer.

Because many organic chemicals are not appreciably water-soluble, DMSO is used routinely as the solvent for the test chemical. Other solvents that are used occasionally are ethanol, benzene, and water. Several doses of the test chemical (up to 5%, w/v or v/v) are tested in each experiment, and appropriate controls are included.

The suspension mixture is incubated at 30° C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiological saline, and a volume of 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on plates containing the same ingredients as the broth plus 1.5% agar; five plates are used for the 10^{-3} dilution and three plates are used for the 10^{-5} dilution. The plates are incubated for 2 days at 30° C, followed by 2 days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the 10^{-3} dilution are scanned with a dissecting microscope at 10 X magnification, and the number of mitotic recombinants (red colonies or red sectors) is recorded. The surviving fraction of organisms is determined from the total number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per millimeter as well as in the relative number of mitotic recombinants per 10^5 survivors.

RESULTS AND DISCUSSION

Tables 1 and 2 present the results of testing T-2379 Du in the Ames Salmonella/microsome assay. The data in each table are the results from one assay conducted in duplicate (two plates per strain per dose); each assay was performed on a separate day. T-2379 Du was tested over a concentration range of 10 to 5000 µg/plate, both with and without metabolic activation. T-2379 Du formed a precipitate when it was added to the top agar at 1000 and 5000 µg/plate (Table 1) and 500, 1000, and 5000 µg/plate (Table 2). No dose-related increase in mutants over the background number was observed; therefore, we conclude that T-2379 Du was not mutagenic in S. typhimurium.

Tables 3 and 4 present the results of testing T-2380 Du. The compound was tested over a concentration range of 10 to 5000 µg/plate. A precipitate formed at concentrations of 500 µg/plate and higher. A dose-related increase in mutants over the background count was observed in S. typhimurium strain TA100 with metabolic activation in both experiments. However, mutagenicity was not observed in strains TA1535, TA1537, TA1538, or TA98 with or without metabolic activation or in strain TA100 without activation.

The results of the assays of T-2379 Du with S. cerevisiae D3 are shown in Tables 5 and 6. T-2379 Du was tested at concentrations from 0.1 to 5.0% (Table 5) and 0.5 to 5.0% (Table 6) both with and without metabolic activation. An apparent recombinogenic response was observed at 0.5% with metabolic activation (Table 6), but this effect was not reproducible and not dose-related. Therefore, we do not believe that the data should be interpreted as indicating a recombinogenic effect.

Tables 7 and 8 present the results of testing T-2380 Du with S. cerevisiae D3. T-2380 Du was tested once at concentrations of 0.1 to 5.0% (Table 7) and once at concentrations of 0.5 to 5.0% (Table 8). An apparent recombinogenic response was observed at 1.0% both with and without metabolic activation (Table 7). However, this effect was not

reproducible (Table 8). Therefore, we do not believe that the data should be interpreted as indicating a true recombinogenic effect.

In conclusion, T-2379 Du was neither mutagenic in S. typhimurium nor recombinogenic in S. cerevisiae. T-2380 Du was mutagenic in assays with S. typhimurium strain TA100 when the metabolic activation system was present. It was not mutagenic with any of the other Salmonella strains, and it was not recombinogenic in S. cerevisiae D3.

Table 1
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 T-2379 Du

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|-------------------------|----------------------|--|--------------------------------|--------|----------|----------|----------|
| | | | TA1535 | TA1537 | TA1538 | TA100 | |
| Negative control (DMSO) | - | | 33, 19 | 18, 5 | 17, 15 | 28, C* | 112, 122 |
| | + | | 17, 15 | 15, 17 | 19, 17 | 32, 32 | 104, 102 |
| Positive controls | - | 1.0 | 514, 513 | | | | 512, 565 |
| | - | 50 | 152, 136 | | | | |
| 9-Aminoacridine | - | 5.0 | | | 466, 387 | 320, 264 | |
| 2-Nitrofluorene | - | 1.0 | | | 9, 14 | 24, 19 | 126, 104 |
| 2-Anthramine | + | 1.0 | | | 322, 344 | 284, 238 | 337, 383 |
| | - | 2.5 | 31, 30 | 13, 12 | | | |
| | + | 2.5 | 155, 149 | 97, 85 | | | |
| T-2379 Du | - | 10 | 35, 30 | 12, 7 | 12, 6 | 24, 24 | 101, 115 |
| | - | 50 | 26, 32 | 2, 13 | 9, 10 | 19, 21 | 98, 114 |
| | - | 100 | 31, 32 | 5, 8 | 5, 6 | 15, 20 | 93, 89 |
| | - | 500 | 30, 21 | 7, 3 | 6, 5 | 18, 9 | 72, 62 |
| | - | 1000† | 20, 15 | 4, 2 | 4, 3 | 8, 19 | 78, 77 |
| | - | 5000† | 18, 20 | 4, 3 | 4, 2 | 13, 9 | 72, 62 |
| | + | 10 | 8, 14 | 10, 9 | 10, 12 | 21, 26 | 89, 85 |
| | + | 50 | 15, 7 | 8, 10 | 11, 14 | 27, 26 | 89, 108 |
| | + | 100 | 9, 12 | 9, 15 | 5, 12 | 29, 20 | 112, 112 |
| | + | 500 | 8, 6 | 9, 9 | 15, 21 | 26, 31 | 109, 96 |
| + | 1000† | 9, 12 | 7, 7 | 16, 8 | 12, 21 | 96, 87 | |
| + | 5000† | 5, 13 | 5, 8 | 9, 13 | 12, 17 | 72, 71 | |

* C, contaminated.
 † Precipitates formed at these concentrations.

Table 2
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2379 Du

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|-------------------------|-------------------------|--|--------------------------------|---------|----------|----------|----------|
| | | | TA1535 | TA1537 | TA1538 | TA100 | |
| Negative control (DMSO) | - | | 53, 63 | 9, 13 | 8, 9 | 19, 25 | 117, 104 |
| | + | | 20, 9 | 15, 11 | 19, 16 | 32, 30 | 125, 113 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1.0 | 423 | 446 | | | 419, 490 |
| 9-Aminoacridine | - | 50 | | 103, 65 | | | |
| 2-Nitrofluorene | - | 1.0 | | | 456, 453 | 251, 354 | |
| 2-Anthramine | + | 1.0 | | | 12, 15 | 27, 31 | 142, 145 |
| | - | 1.0 | 49, 51 | 12, 11 | 231, 269 | 186, 179 | 257, 335 |
| | - | 2.5 | 102, 97 | 44, 40 | | | |
| | + | 2.5 | | | | | |
| T-2379 Du | - | 10 | 45, 44 | 9, 6 | 13, 14 | 19, 20 | 115, 109 |
| | - | 50 | 43, 60 | 9, 8 | 6, 7 | 23, 18 | 104, 105 |
| | - | 100 | 43, 53 | 7, 7 | 6, 7 | 18, 18 | 111, 105 |
| | - | 500* | 19, 52 | 4, 2 | 9, 10 | 10, 7 | 61, 80 |
| | - | 1000* | 32, 34 | 1, 1 | 10, 9 | 22, 2 | 58, 85 |
| | - | 5000* | 45, 46 | 10, 6 | 11, 11 | 18, 15 | 91, 93 |
| | + | 10 | 14, 19 | 6, 13 | 17, 21 | 41, 33 | 124, 89 |
| | + | 50 | 16, 15 | 17, 18 | 17, 18 | 24, 52 | 129, 131 |
| | + | 100 | 20, 11 | 18, 17 | 23, 24 | 25, 41 | 131, 108 |
| | + | 500* | 11, 14 | 13, 7 | 17, 19 | 30, 31 | 111, 106 |
| | + | 1000* | 15, 8 | 5, 12 | 21, 9 | 34, 25 | 103, 112 |
| | + | 5000* | 10, 40 | 5, 15 | 14, 15 | 24, 27 | 92, 80 |

* Precipitates formed at these concentrations.

Table 3
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2380 Du

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|-------------------------|----------------------|--|--------------------------------|--------|----------|----------|----------|
| | | | TA1535 | TA1537 | TA1538 | TA100 | |
| Negative control (DMSO) | - | | 33, 19 | 18, 5 | 17, 15 | 28, C* | 112, 122 |
| | + | | 17, 15 | 15, 17 | 19, 17 | 32, 32 | 104, 102 |
| Positive controls | - | 1.0 | 514, 513 | | | | 512, 565 |
| | - | 50 | 152, 136 | | | | |
| Sodium azide | - | 5.0 | | | 466, 387 | 320, 264 | |
| 9-Aminoacridine | - | 1.0 | | | 9, 14 | 24, 19 | 126, 104 |
| 2-Nitrofluorene | - | 1.0 | | | 322, 344 | 284, 238 | 337, 383 |
| 2-Anthramine | + | 1.0 | 31, 30 | 13, 12 | | | |
| | - | 2.5 | 155, 149 | 97, 85 | | | |
| T-2380 Du | - | 10 | 31, 34 | 8, 8 | 21, 5 | 19, 33 | 84, 76 |
| | - | 50 | 25, 31 | 6, 5 | 6, 17 | 32, 30 | 66, 81 |
| | - | 100 | 25, 32 | 5, 13 | 8, 8 | 31, 24 | 96, 114 |
| | - | 500* | 29, 26 | 4, 11 | 7, 13 | 27, 20 | 78, 97 |
| | - | 1000* | 32, 24 | 7, 8 | 9, 14 | 18, 10 | 70, 89 |
| | - | 5000* | 27, 16 | 8, 5 | 7, 10 | 18, 24 | 67, 94 |
| | + | 10 | 13, 19 | 16, 16 | 22, 29 | 32, 27 | 66, 109 |
| | + | 50 | 14, 12 | 17, 16 | 30, 28 | 37, 37 | 109, 111 |
| | + | 100 | 20, 12 | 12, 15 | 18, 28 | 29, 39 | 124, 139 |
| | + | 500* | 10, 12 | 14, 20 | 19, 15 | 31, 38 | 179, 184 |
| | + | 1000* | 9, 3 | 13, 16 | 20, 19 | 30, 30 | 188, 195 |
| | + | 5000* | 11, 9 | 7, 15 | 12, 14 | 36, 31 | 262, 265 |

* Precipitates formed at these concentrations.

Table 4
IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2380 Du

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|-------------------------|----------------------|--|--------------------------------|---------|----------|----------|----------|
| | | | TA1535 | TA1537 | TA1538 | TA100 | |
| Negative control (DMSO) | - | | 53, 63 | 9, 13 | 14, 11 | 19, 25 | 129, 153 |
| | + | | 20, 9 | 15, 11 | 19, 18 | 32, 30 | 113, 93 |
| Positive controls | | 1.0 | 423, 446 | 103, 65 | | | 531, 406 |
| Sodium azide | - | 50 | | | 562, 540 | 251, 354 | |
| 9-Aminoacridine | - | 5.0 | | | 15, 9 | 27, 31 | 112, 104 |
| 2-Nitrofluorene | - | 1.0 | | | 280, 316 | 186, 179 | 348, 306 |
| 2-Anthramine | + | 1.0 | | | | | |
| | - | 2.5 | 49, 51 | 12, 11 | | | |
| | + | 2.5 | 102, 97 | 44, 40 | | | |
| | - | 10 | 36, 49 | 7, 8 | 9, 10 | 18, 26 | 103, 97 |
| | - | 50 | 49, 45 | 7, 8 | 13, 14 | 28, 24 | 97, 126 |
| | - | 100 | 37, 50 | 7, 5 | 13, 15 | 21, 24 | 125, 103 |
| | - | 500* | 44, 48 | 7, 4 | 6, 18 | 26, 28 | 113, 100 |
| | - | 1000* | 31, 20 | 6, 4 | 10, 6 | 27, 27 | 98, 113 |
| | - | 5000* | 28, 40 | 6, 2 | 6, 10 | 17, 32 | 96, 113 |
| | + | 10 | 9, 16 | 8, 8 | 21, 28 | 31, 29 | 75, 85 |
| | + | 50 | 15, 20 | 7, 6 | 15, 24 | 49, 37 | 84, 112 |
| | + | 100 | 12, 20 | 13, 5 | 19, 19 | 52, 41 | 111, 136 |
| | + | 500* | 20, 19 | 16, 4 | 21, 29 | 31, 39 | 172, 130 |
| | + | 1000* | 14, 9 | 13, 7 | 14, 20 | 35, 31 | 179, 190 |
| | + | 5000* | 14, 13 | 7, 10 | 14, 15 | 52, 33 | 133, 153 |

T-2380 Du

* Precipitates formed at these concentrations.

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Table 5

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
T-2379 Du

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|-------------------------|--|--------------------------------------|---------|--------------------------------|-------------------------|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-5}$) | Per 10^5 Survivors |
| Negative control (DMSO) | - | | 6.7 | 100 | 1.5 | 2.2 |
| | + | | 8.2 | 100 | 2.5 | 3.1 |
| Positive control 1,2,3,4-Diepoxybutane | - | 0.025 | 6.5 | 97 | 465 | 720 |
| | + | 0.025 | 8.0 | 98 | 365 | 460 |
| T-2379 Du | - | 0.1 | 2.8 | 42 | 1.0 | 3.6 |
| | - | 0.5 | 5.5 | 82 | 5.0 | 9.1 |
| | - | 1.0 | 6.0 | 90 | 5.0 | 8.3 |
| | - | 5.0 | 6.4 | 96 | 4.0 | 6.3 |
| | + | 0.1 | 11.3 | 138 | 2.0 | 1.8 |
| | + | 0.5 | 6.7 | 82 | 3.0 | 4.5 |
| | + | 1.0 | 5.5 | 67 | -0- | -0- |
| | + | 5.0 | 6.7 | 82 | 1.0 | 1.5 |

Table 6

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE

T-2379 Du

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|----------------------|------------------------------------|-----------------------------------|---------|-----------------------------|----------------------|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per 10^5 Survivors |
| Negative control (DMSO) | - | | 7.2 | 100 | 4.0 | 5.6 |
| | + | | 7.2 | 100 | 3.5 | 4.9 |
| Positive control 1,2,3,4-Diepoxybutane | - | 0.025 | 7.0 | 97 | 550 | 790 |
| | + | 0.025 | 7.7 | 107 | 595 | 770 |
| T-2379 Du | - | 0.5 | 6.7 | 93 | 2.0 | 3.0 |
| | - | 0.75 | 6.7 | 93 | 6.0 | 9.0 |
| | - | 1.0 | 7.5 | 104 | 2.0 | 2.7 |
| | - | 2.5 | 7.4 | 103 | 9.0 | 12 |
| | - | 5.0 | 8.2 | 114 | 6.0 | 7.3 |
| | + | 0.5 | 3.6 | 50 | 6.0 | 17 |
| | + | 0.75 | 5.1 | 71 | 5.0 | 9.8 |
| | + | 1.0 | 3.7 | 51 | 3.0 | 8.1 |
| + | 2.5 | 9.1 | 126 | 3.0 | 3.3 | |
| + | 5.0 | 9.0 | 125 | 6.0 | 6.7 | |

Table 7

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
T-2380 Du

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|-------------------------|--|--------------------------------------|---------|--------------------------------|-------------------------|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per 10^5 Survivors |
| Negative control (DMSO) | - | | 6.7 | 100 | 1.5 | 2.2 |
| | + | | 8.2 | 100 | 2.5 | 3.1 |
| Positive control 1,2,3,4-Diepoxybutane | - | 0.025 | 6.5 | 97 | 465 | 720 |
| | + | 0.025 | 8.0 | 98 | 365 | 460 |
| T-2380 Du | - | 0.1 | 10.2 | 152 | 1.0 | 1.0 |
| | - | 0.5 | 8.8 | 131 | 3.0 | 3.4 |
| | - | 1.0 | 6.3 | 94 | 5.0 | 7.9 |
| | - | 5.0 | 6.5 | 97 | 3.0 | 4.6 |
| | + | 0.1 | 6.8 | 83 | 4.0 | 5.9 |
| | + | 0.5 | 7.5 | 91 | 1.0 | 1.3 |
| | + | 1.0 | 7.0 | 85 | 7.0 | 10 |
| | + | 5.0 | 4.9 | 60 | 3.0 | 6.1 |

Table 8

IN VITRO ASSAYS WITH SAGCHAROMYCES CEREVISIAE
T-2380 Du

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|-------------------------|--|--------------------------------------|---------|--------------------------------|-----------|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Survivors |
| Negative control (DMSO) | - | | 7.2 | 100 | 4.0 | 5.6 |
| | + | | 7.2 | 100 | 3.5 | 4.9 |
| Positive control 1,2,3,4-Diepoxybutane | - | 0.025 | 7.0 | 97 | 550 | 790 |
| | + | 0.025 | 7.7 | 107 | 595 | 770 |
| T-2380 Du | - | 0.5 | 6.5 | 90 | 1.0 | 1.5 |
| | - | 0.75 | 6.9 | 96 | 2.0 | 2.9 |
| | - | 1.0 | 6.7 | 93 | 2.0 | 3.0 |
| | - | 2.5 | 7.0 | 97 | 4.0 | 5.7 |
| | - | 5.0 | 8.0 | 111 | 6.0 | 7.5 |
| | + | 0.5 | 6.4 | 89 | 3.0 | 4.7 |
| | + | 0.75 | 6.5 | 90 | 2.0 | 3.1 |
| | + | 1.0 | 7.0 | 97 | 1.0 | 1.4 |
| | + | 2.5 | 7.1 | 99 | 3.0 | 4.2 |
| | + | 5.0 | 7.8 | 108 | 2.0 | 2.6 |

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