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MUTAGENICTY EVALUATION OF 10055-49-1 IN THE MOUSE LYMPHOMA ASSAY (FINAL) WITH COVER LETTER

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CONTAINS NO CBI

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These reports are being submitted for:

W. R. Grace & Co.-Conn.
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Columbia, MD 21044

Sincerely,

J. W. Raksis

A:\JR91-013/lw

Attachments - 20

8691000063B

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FHP 3000

Toluene Diisocyanate
26471-62-5

# MUTAGENICITY EVALUATION

# OF

# 10055-49-1

# IN THE MOUSE LYMPHOMA ASSAY

# FINAL REPORT

SUBMITTED TO

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MAY, 1977

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Litton BIONETICS

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## EVALUATION SUMMARY

The test compound, 10055-49-1, was evaluated as being nonmutagenic at the TK locus of L5178Y mouse lymphoma cells when evaluated under the conditions of this assay.

SPONSOR: W.R. Grace & Co.

MATERIAL: 10055-49-1

SUBJECT: FINAL REPORT L5178Y MOUSE LYMPHOMA MUTAGENICITY ASSAY

1. OBJECTIVE

The objective of this study was to evaluate 10055-49-1 for specific locus forward mutation induction in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay.

2. MATERIALS

A. Test Compound

1. Date Received: March 25, 1977

2. Description: Very viscous amber liquid

B. Indicator Cells

The Fischer mouse lymphoma cell line used in this study was derived from L5178Y. The cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine (BUdR) sensitive. Scoring for mutation was based on selecting cells that have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BUdR.

C. Media

The cells were maintained in Fischer's Medium for Leukemic Cells of Mice with 10% horse serum and sodium pyruvate. Cloning medium consisted of Fischer's medium with 20% horse serum, sodium pyruvate, and 0.37% agar. Selection medium was made from cloning medium by the addition of 5.0 mg of BUdR to 100 ml of cloning medium.

D. Control Compounds

1. Negative Control

The solvent in which the test compound was dissolved was used as a negative control and is designated as solvent control in the data table. The actual solvent is listed in the Results Section.

2. MATERIALS (Continued)

D. Control Compounds

2. Positive Controls

Ethylmethanesulfonate (EMS), which induces mutation by base-pair substitution, was dissolved in culture medium and used as a positive control for the nonactivation studies at a final concentration of 0.600 µl/ml.

Dimethylnitrosamine (DMN), which requires metabolic biotransformation by microsomal enzymes, was used as a positive control substance for the activation studies at a final concentration of 0.300 µl/ml.

3. EXPERIMENTAL DESIGN

A. Toxicity

The solubility, toxicity, and doses for all chemicals were determined prior to screening. The effect of each chemical on the survival of the indicator cells was determined by exposing the cells to a wide range of chemical concentrations in complete growth medium. Toxicity was measured as loss in growth potential of the cells induced by a five-hour exposure to the chemical followed by a 24-hour expression period in growth medium. A minimum of four doses was selected from the range of concentration by using the highest dose that showed no loss in growth potential as the penultimate dose and by bracketing this with one higher dose and at least two lower doses. Toxicity produced by chemical treatment was monitored during the experiment.

B. Assays

1. Nonactivation Assay

The procedure used is a modification of that reported by Clive and Spector (Mutation Research, 31:17-29, 1975). Prior to each treatment, cells were cleansed of spontaneous TK-/- by growing them in a medium containing thymidine, hypoxanthine, methotrexate, and glycine (THMG). This medium permits the survival of only those cells that produce the enzyme thymidine kinase, and can therefore utilize the exogenous thymidine from the medium. The test compound was added to the cleansed cells in growth medium at the predetermined doses for five hours. The mutagenized cells were washed, fed, and allowed to express in growth medium for three days. At the end of this expression period, TK-/- mutants were detected by cloning the cells in the selection medium for ten days. Surviving cell populations were determined by plating diluted aliquots in nonselective growth medium.

3. EXPERIMENTAL DESIGN (Continued)

B. Assays

2. Activation Assay

The activation assay differs from the nonactivation assay in the following manner only. Two milliliters of the reaction mixture were added to 10 ml of growth medium. The desired number of cleansed cells was added to this mixture, and the flask was incubated on a rotary shaker for five hours. The incubation period was terminated by washing the cells twice with growth medium. The washed mutagenized cells were then allowed to express for three days and were cloned as indicated for the nonactivated cells.

C. Preparation of 9,000 x g Supernatant

Male random bred mice were killed by cranial blow, decapitated, and bled. The liver was immediately dissected from the animal using aseptic technique and placed in ice-cold 0.25M sucrose buffered with Tris buffer at a pH of 7.4. When an adequate number of livers had been collected, they were washed twice with fresh buffered sucrose and completely homogenized. The homogenate was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from this centrifuged sample was retained and frozen at -80C until used in the activation system. This microsome preparation was added to a "core" reaction mixture to form the activation system described below:

| Component | Final Concentration/ml |
|---|---|
| 1. TPN (sodium salt) | 6 umoles |
| 2. Isocitric acid | 35 umoles |
| 3. Tris buffer, pH 7.4 | 28 umoles |
| 4. $MgCl_2$ | 2 umoles |
| 5. Homogenate fraction equivalent to 25 mg of wet tissue | |

D. Screening

A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. Colonies were counted on an electronic colony counter that resolves all colonies greater than 200 microns in diameter.

4. RESULTS

The data presented in the following table show the concentrations of the test compound employed, the number of mutant clones obtained, the surviving populations after the expression period, and the calculated mutation frequencies.

4. SUMMARY OF MOUSE LYMPHOMA (L5178Y) RESULTS LITTON BIONETICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 10055-49-1

B. SOLVENT: DMSO
C. TEST DATE: MAY 10, 1977
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) OR NANOLITERS (NL) PER MILLILITER.

| TEST | S-9 TISSUE | S-9 SOURCE | DAILY COUNTS (CELLS/ML X 10E5) 1 | 2 | 3 | RELATIVE SUSPENSION GROWTH (% OF CONTROL) | MUTANT CLONES | VIABLE CLONES | RELATIVE CLONING EFFICIENCY (% OF CONTROL) | PERCENT RELATIVE GROWTH* | MUTANT FREQUENCY** (X 10E-4) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NONACTIVATION | | | | | | | | | | | |
| SOLVENT CONTROL | --- | --- | 5.2 | 8.3 | 10.1 | 100.0 | 28.0 | 206.0 | 100.0 | 100.0 | 0.1359 |
| NEGATIVE CONTROL | --- | --- | 5.1 | 11.4 | 7.0 | 93.4 | 49.0 | 196.0 | 95.1 | 88.8 | 0.2500 |
| EMS .600UL/ML | --- | --- | 4.0 | 3.5 | 6.7 | 36.3 | 425.0 | 97.0 | 47.1 | 17.1 | 4.3814 |
| TEST COMPOUND | | | | | | | | | | | |
| 0.08000 UL/ML | --- | --- | 4.4 | 12.8 | 6.0 | 77.5 | 8.0 | 215.0 | 104.4 | 80.9 | 0.0372 |
| 0.16000 UL/ML | --- | --- | 6.4 | 10.0 | 7.2 | 105.7 | 26.0 | 176.0 | 85.4 | 90.3 | 0.1477 |
| 0.32000 UL/ML | --- | --- | 6.6 | 7.6 | 9.6 | 110.5 | 24.0 | 226.0 | 109.7 | 121.2 | 0.1062 |
| 0.65000 UL/ML | --- | --- | 7.0 | 8.6 | 10.8 | 149.1 | 44.0 | 105.0 | 51.0 | 76.0 | 0.4190 |
| 1.25000 UL/ML | --- | --- | 7.8 | 6.8 | 7.6 | 92.5 | 32.0 | 214.0 | 103.9 | 96.1 | 0.1495 |
| ACTIVATION | | | | | | | | | | | |
| SOLVENT CONTROL | MOUSE | LIVER | 6.1 | 6.4 | 6.8 | 100.0 | 16.0 | 347.0 | 100.0 | 100.0 | 0.0461 |
| NEGATIVE CONTROL | MOUSE | LIVER | 7.3 | 6.8 | 9.2 | 172.0 | 31.0 | 250.0 | 72.0 | 123.9 | 0.1240 |
| DMN .300UL/ML | MOUSE | LIVER | 3.3 | 4.1 | 3.4 | 15.6 | 209.0 | 38.0 | 11.0 | 1.7 | 5.5000 |
| TEST COMPOUND | | | | | | | | | | | |
| 0.08000 UL/ML | MOUSE | LIVER | 6.0 | 7.6 | 5.2 | 89.3 | 28.0 | 194.0 | 55.9 | 49.9 | 0.1443 |
| 0.16000 UL/ML | MOUSE | LIVER | 6.0 | 10.8 | 11.2 | 273.4 | 41.0 | 205.0 | 59.1 | 161.5 | 0.2000 |
| 0.32000 UL/ML | MOUSE | LIVER | 8.0 | 9.4 | 6.0 | 170.0 | 48.0 | 252.0 | 72.6 | 123.4 | 0.1905 |
| 0.65000 UL/ML | MOUSE | LIVER | 7.2 | 7.2 | 8.6 | 167.9 | 26.0 | 216.0 | 62.2 | 104.5 | 0.1204 |
| 1.25000 UL/ML | MOUSE | LIVER | 6.4 | 11.0 | 8.6 | 228.1 | 36.0 | 222.0 | 64.0 | 145.9 | 0.1622 |

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** (MUTANT CLONES / VIABLE CLONES) X 10E-4

## 5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, 10055-49-1, was evaluated for genetic activity in the L5178Y mouse lymphoma cell line. The genotype of this cell line is TK+/- and will detect forward mutation to TK-/- cells that are resistant to BUdR. The following results were obtained:

### A. Solubility and Toxicity Results

The test compound was soluble in DMSO at 100 µl/ml when warmed to 37C. Serial two-fold dilutions were made of this stock using DMSO as the diluent. One-tenth milliliter of the diluted stock solutions was added to the cells in 10 ml of growth medium to yield final concentrations in the range of 0.001 µl/ml to 1 µl/ml. The final solutions were cloudy at 0.032 µl/ml and higher but no precipitate formed. No toxicity was observed at these levels.

### B. Positive and Negative Control Results

DMSO at a concentration of 1% (equivalent to the amount used in the treatment levels) was used as the solvent control compound. No genetic effects were attributable to the DMSO at this concentration.

The positive control mutagens, EMS and DMN gave positve responses within the acceptable range.

### C. Test Chemical Results

Based on the results of the toxicity study, cells were exposed to the test compound in the concentration range of 0.075 µl/ml to 1.25 µl/ml as indicated in the data summary table. The results appeared negative since none of the dose-related patterns associated with mutagenesis were observed. An elevated mutation frequency was associated with the 0.625 µl/ml dose in the nonactivation series, but can be ascribed to random variation due mainly to a low viable colony count associated with this concentration. Although the mutation frequencies of two of the concentrations of the activation series are nearly four times the solvent control value, none of the expected dose-related patterns were present and it was concluded that the elevated values did not reflect a mutagenic response, but rather that they were the result of a solvent control value 1/3 to 1/4 of the anticipated frequency.

5. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

D. Conclusions

The test compound, 10055-49-1, did not meet our criteria for induced mutagens and has been evaluated as being nonmutagenic at the TK locus in L5178Y mouse lymphoma cells under the conditions of this test.

Submitted by:

6/3/77

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Director
Department of Genetics

Reviewed by:

6/3/77

Robert J. Weir, Ph.D. Date
Vice President

## 6. CRITERIA USED IN THE EVALUATION

Several criteria have been established which, if met, provide a basis for declaring a material genetically active in the Mouse Lymphoma Assay. These criteria are derived from a historical data base and are helpful in maintaining uniformity in evaluations from material to material and run to run. While these criteria are reasonably objective, a certain amount of flexibility may be required in making the final evaluation since absolute criteria may not be applicable to all biological data.

A compound is considered mutagenic in the Mouse Lymphoma Assay if:

a. A dose response relationship is observed over three of the four dose levels employed.

b. The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value.

c. The solvent control data are within the normal range of the spontaneous background for the TK locus.

All evaluations of mutagenic activity are based on the concurrent solvent control value run with the experiment in question. Positive control values are not used as reference points, but are included to ensure the current cell population responds to direct and promutagens under the appropriate treatment conditions.

Occasionally, a single point within a concentration range will show an increase 2.5 times greater than the spontaneous background. If the increase is at the high dose, is reproducible, and if an additional higher dose level is not feasible because of toxicity, the chemical can be considered mutagenic. If the increase is internal within the dose range and is not reproducible, the increase will normally be considered aberrant. If the internal increase is reproducible, several doses clustered around the positive concentration will be examined to either confirm or reject the reliability of the effect.

As the data base on the assay increases, the evaluation criteria can be expected to become more firmly established.

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