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FMC



84948888261

Sanju Biwan
Control Inc.
1 Central Plaza
11300 Rockwell Pike
Rockwell, MD 20852

Contains No GSI

Dear Sanju:

Enclosed is the information you requested on the mutagenic screen for Methallyl Chloride. Please let me know if there are any additional questions.

Sincerely,

Joseph G. Acker,
Manager
Manufacturing

r12B1
tmb23

cc: H. Latourette

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received 8/6/87

MUTAGENICITY EVALUATION
OF
MR S490 METHALLYL CHLORIDE
FINAL REPORT
ACT 049.61

SUBMITTED TO
FMC CORPORATION
AGRICULTURAL CHEMICAL DIVISION
MIDDLEPORT, NEW YORK 14105

SUBMITTED BY
LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795
LBI PROJECT NO. 2547
JUNE 22, 1976



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SPONSOR: FNC Corporation

MATERIAL: NR S/90 methallyl chloride

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: May 25, 1976
2. Description: Clear liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

1. Yeast Strain: Saccharomyces cerevisiae, stra D4
2. Bacteria Strains: Salmonella typhimurium, strains:
TA-1535 TA-98
TA-1537 TA-100
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 μ moles
2. Isocitric acid	35 μ moles
3. Tris buffer, pH 7.4	28 μ moles
4. MgCl ₂	2 μ moles
5. Homogenate fraction equivalent to 25 mg of wet tissue	



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2. MATERIALS (Continued)

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from the livers of Sprague-Dawley adult male rats. The animals were pretreated with Aroclor 1254 (500 mg/kg) 5 days before kill.

E. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL*</u>	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS**
	2-Nitrofluorene (NF)	Dimethylsulfoxide***	FS**
	Quinacrine mustard (QM)	Water or saline	FS**
Activation	2-Anthramine (A ₁ YTH)	Dimethylsulfoxide***	BPS**
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide***	FS**
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide***	FS**
	Dimethylnitrosamine (DMNA)	Saline	BPS**

* Concentrations given in Results Section

** BPS = Base-pair substitution

FS = Frameshift

*** Previously shown to be nonmutagenic



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3. EXPERIMENTAL DESIGN

A. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated, and bled. Organs were immediately dissected from the animal using aseptic techniques and were placed in ice-cold 0.25 M sucrose buffered with Tris at a pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4C. The organ homogenate obtained from this step was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80C. Samples from this preparation were used for the activation studies.

B. Plate Test (Overlay Method)

Approximately 10^9 cells from a log phase culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Four dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

C. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points.



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4. SUMMARY OF PLATE TEST RESULTS

- A. Name or code designation of the test compound: MR S490 methallyl chloride
- B. Solvent: DMSO
- C. Test date: May 26, 1976
- D. Concentrations of the test compound: (1) 0.01 μ l (2) 0.1 μ l (3) 1 μ l (4) 5 μ l (5) 10 μ l

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE					
			TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4*
<u>NONACTIVATION</u>								
Solvent control	---	---	64	19	28	38	178	50
Positive control**	---	---	>10 ³	>10 ³	>10 ³	433	305	101
Test compound (1) (2) (3) (4) (5)	---	---	82	11	34	42	144	58
	---	---	96	12	22	44	165	39
	---	---	81	13	24	25	136	55
	---	---	97	17	28	38	163	41
	---	---	--	--	--	--	--	--
<u>ACTIVATION</u>								
Solvent Control	Rat	Liver	66	31	36	50	139	35
Positive control***	Rat	Liver	335	200	>10 ³	>10 ³	200	44
Test compound (1) (2) (3) (4) (5)	Rat	Liver	91	25	47	42	105	38
	Rat	Liver	160	27	37	55	135	35
	Rat	Liver	116	29	51	40	129	36
	Rat	Liver	133	25	50	47	127	31
	Rat	Liver	--	--	--	--	--	--

* Try⁺ revertants per plate

** TA-1535 MNNG 10 μ l/plate
 TA-1537 QM 10 μ l/plate
 TA-1538 NF 100 μ g/plate
 TA-98 NF 100 μ g/plate
 TA-100 MNNG 10 μ l/plate
 D4 MNNG 10 μ l/plate

*** TA-1535 ANTH 100 μ g/plate
 TA-1537 ANQ 100 μ g/plate
 TA-1538 AAF 100 μ g/plate
 TA-98 AAF 100 μ g/plate
 TA-100 ANTH 100 μ g/plate
 D4 DNA 100 μ moles/plate

3. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, NR 5490 methallyl chloride, was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested over a series of concentrations such that there was evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.01 μ l to 5 μ l per plate. A higher dose of 10 μ l per plate was used in the activation test with strain TA-1535.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative. In the test with strain TA-1535, doses 2, 3, and 4 were repeated because of increased mutant frequencies in the initial tests. Because these increases occurred at the higher doses, an additional dose level of 10 μ l per plate was used. The repeat test was negative. The initial results were therefore considered spurious and not the result of mutagenic activity by the test agent.



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5. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

D. Conclusions

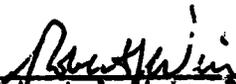
The test compound, MR 5490 methallyl chloride, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:



David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:



Robert J. Meir, Ph.D.
Vice President



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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.



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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Interpretation of Results

The demonstration of dose-related increases in mutant counts is the most reliable method to demonstrate mutagenicity. Mutant increases at only one or two doses may be significant if they occur at the higher doses. Increases at low or intermediate concentrations followed by reduced mutant counts at higher doses may indicate that the test chemical has a narrow activity range, or that the high dose levels were toxic and the induced revertant cells were killed. We are able to detect the latter possibility by inspecting the background growth, and the former possibility can be investigated by looking at a narrow series of dose levels bracketing the presumptive active range.

It is difficult to detect mutagens with little or no toxicity in this assay since such agents are generally weak mutagens and produce only two to threefold increases in mutant counts. Variations of two to threefold are often within normal fluctuations of the spontaneous counts, and the use of even higher concentrations is often difficult because of the likelihood of overloading the system with large quantities of the chemical. To resolve the mutagenicity of such a chemical, other assays to which statistical evaluations can be applied may be necessary.



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