Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

3M COMPANY
REVERSE MUTATION STUDIES WITH
T-1485
IN FIVE SALMONELLA STRAINS AND
ONE SACCHAROMYCES STRAIN

P.O. NO. LP-003397-400

AUGUST 10, 1976

IBT NO. 8540-09238

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NORTHBROOK, ILLINOIS 60062

August 10, 1976

Dr. James E. Long
Manager, Toxicology Services
3M Company
3M Center
St. Paul, Minnesota 55101

Dear Dr. Long:

Re: IBT No. 8540-09238 - Reverse Mutation Studies with T-1485 in Five Salmonella Strains and One Saccharomyces Strain - P.O. No. LP-003397-400

We are submitting herewith our laboratory report prepared in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:bp

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REVERSE MUTATION STUDIES WITH T-1485 IN FIVE SALMONELLA STRAINS AND ONE SACCHAROMYCES STRAIN

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I. Introduction

A sample identified as T-1485 was received from 3M Company for the purpose of conducting reverse mutation studies. The test material was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. This report presents the results of the investigation.

II. Summary

The test material, T-1485, was examined for mutagenic activity in a series of in vitro microbial assays using Salmonella and Saccharomyces indicator organisms. The test material was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. Dose levels of 0.25, 0.5, 5, and 50 µg of T-1485 per plate were used in both the nonactivation and the activation tests. Some physiological effect was observed at the highest dose level (50 µg/plate). The lower dose levels were below toxic levels.

The results obtained with the positive control materials demonstrate that the test systems are functional with known mutagens.

The results of the tests conducted with T-1485, both in the absence and in the presence of the rat liver activation system, were all negative.

It was concluded that T-1485 did not exhibit genetic activity in any of the in vitro assays employed in this investigation.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: Gerald L. Kennedy

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III. Procedure

A. Indicator Microorganisms

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

1. Yeast Strain: Saccharomyces cerevisiae, strain

   D4

2. Bacteria Strains: Salmonella typhimurium, strains

   TA-1535
   TA-1537
   TA-1538
   TA-98
   TA-100

B. Preparation of Liver Homogenate and 9,000 x g Cell Fraction

The tissue homogenate and 9,000 x g cell fraction were prepared from the livers of adult male Sprague-Dawley rats. The animals were treated with 500 mg/kg of Aroclor 1254 five days before kill. A sufficient number of animals to provide the necessary quantities of liver were killed by cranial blow, decapitated, and exsanguinated. The liver was immediately dissected from each animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at a pH of 7.4. Upon collection of the desired quantity of livers, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4C. The liver 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 -80°C. Samples from this preparation were used for the activation tests.

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

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

D. 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 nonactivation tests, the 4 dose levels of the test material 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 (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 37°C, and scored for the number of colonies growing on each plate. Positive and solvent controls were run with each assay.

E. Positive Control Chemicals

Positive controls using both directly active positive chemicals and those that require metabolic activation were run with each assay. The following chemicals were used for positive controls in the nonactivation and activation tests:

Test	Chemical*	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS**
	2-Nitrofluorene (NF)	Dimethylsulfoxide***	FS**
	Quinacrine mustard (QM)	Water or Saline	FS**
Activation	2-Anthramine (ANTH)	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

IV. Results

A summary of the plate test results is presented in Table I. The test material was evaluated at a series of dose levels such that the highest level (50 μg/plate) showed some physiological effect. The lower dose levels (0.25, 0.5, and 5 μg/plate) were below toxic levels. The results obtained with the positive control materials demonstrate that the test systems are functional with known mutagens.

The results of the tests conducted with T-1485 were all negative.

TABLE I

TEST MATERIAL: T-1485

Reverse Mutation Studies in Five Salmonella Strains and one Saccharomyces Strain

Summary of Plate Test Results

Test	Group	Test Material	Dose Level (µg/plate)	Species	Tissue	Strain: TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4*
						Number of Revertants per Plate					
Nonactivation	Solvent Control (DMSO)	-	-	-	-	45	7	11	42	113	66
	Positive Control	**	**	-	-	$>10^3$	$>10^3$	$>10^3$	42	$>10^3$	155
	T-I	T-1485	0.25	-	-	77	5	5	46	132	55
	T-II	T-1485	0.5	-	-	78	9	10	46	145	60
	T-III	T-1485	5	-	-	66	5	10	46	108	45
	T-IV	T-1485	50	-	-	75	3	14	18	104	40
Activation	Solvent Control (DMSO)	-	-	Rat	Liver	54	28	20	36	124	37
	Positive Control	***	***	Rat	Liver	485	636	$>10^3$	$>10^3$	520	44
	T-I	T-1485	0.25	Rat	Liver	66	28	20	21	150	36
	T-II	T-1485	0.5	Rat	Liver	62	24	21	27	146	34
	T-III	T-1485	5	Rat	Liver	30	27	21	28	149	35
	T-IV	T-1485	50	Rat	Liver	27	23	20	25	133	32

* Try[1] convertants per plate

**	TA-1535	MNNG	10 µl/plate
	TA-1537	QM	10 µl/plate
	TA-1538	NF	100 µg/plate
	TA-98	NF	100 µg/plate
	TA-100	MNNG	10 µl/plate
	D4	MNNG	10 µl/plate
***	TA-1535	ANTH	100 µg/plate
	TA-1537	AMQ	100 µg/plate
	TA-1538	AAF	100 µg/plate
	TA-98	AAF	100 µg/plate
	TA-100	ANTH	100 µg/plate
	D4	DMNA	100 µmoles/plate