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SRI International

IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY'S COMPOUND T-3752

Final Report

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SUMMARY

SRI International examined 3M Company's Compound T-3752 for mutagenic activity in the standard Ames Salmonella/microsome assay with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium. Compound T-3752 was also screened for recombinogenic activity in the yeast Saccharomyces cerevisiae D3 assay. Both assays were performed in the presence and absence of a rat-liver metabolic activation system. All tests were performed in compliance with the United States Food and Drug Administration (FDA) Good Laboratory Practice Standards.

Compound T-3752 was reproducibly nonmutagenic and nonrecombinogenic when tested according to these procedures.

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QUALITY ASSURANCE UNIT

Final Report Statement

SRI International assures the quality and integrity of this study, In Vitro Microbiological Mutagenecity Assays Of Compound T-3752, for the 3M Company.

Inspections and audits were performed during different phases of the study, and the Study Director and SRI management were notified of the findings of the Quality Assurance Unit.

This report accurately describes the methods and standard operating procedures of the study. Any deviations from the approved protocol were made with the proper authorization and documentation. Any deviations from standard operating procedures were documented.

Manager of Regulatory Affairs
and Quality Assurance

Date

INTRODUCTION

SRI International examined 3M Company's Compound T-3752 for mutagenicity in the standard Ames Salmonella/microsome assay with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium. Compound T-3752 was also tested for recombinogenic activity in the yeast Saccharomyces cerevisiae D3 assay. An Aroclor 1254-stimulated, rat-liver homogenate metabolic activation system was included in the assay procedures to provide metabolic steps that the microorganisms 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. The assay procedure with S. cerevisiae is about 60% reliable in detecting carcinogens as agents that increase mitotic recombination. 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 carcinogenic or noncarcinogenic to man.

Evaluation of experimental results from the Salmonella assay consists of comparing the number of histidine-independent colonies on the treated agar plates with the number observed on the control plates. Because all the plated Salmonella indicator organisms undergo a few cell divisions in the presence of the test chemical, the test is semiquantitative in nature. The plate test procedure does not permit quantitative determination of the number of cells surviving the chemical treatment. It is the demonstration of a mutagenic dose-response relationship that is important in establishing mutagenicity.

The test chemicals are assayed at several dose levels within a non-toxic dose range--with the exception of the highest dose level, which sometimes exhibits toxicity. Toxicity is evidenced by several phenomena: clearing of the background bacterial lawn growth, formation of pinpoint colonies consisting of surviving cells, and a decrease in the number of revertant colonies below the spontaneous background.

A chemical is considered a mutagen in the Salmonella assay if it elicits a reproducible, dose-related increase in the number of histidine revertants per plate in one or more tester strains.

The yeast Saccharomyces cerevisiae D3 is a eukaryotic microorganism capable of detecting mitotic recombination, as expressed through a mutation leading to a defective enzyme in the adenine-metabolizing pathway, resulting in a red-pigmented colony. In this assay, the yeast cells are exposed to several concentrations of the test chemical, usually ranging from a concentration that results in no killing to one that causes 50% killing. Any concentration that induces 90% killing is considered toxic. When the number of genetically altered colonies per milliliter (yield) and the ratio of altered colonies to survivors (frequency) from the treated cells are unequivocally larger than those of the solvent-treated controls, we conclude that the exposure of the cells to the compound induces mitotic recombination. If this event is dose-related, the observation is termed a positive response.

The assays with Compound T-3752 were begun on 24 May 1985 and testing was completed on 14 June 1985. Copies of the final report will be kept in our files (Building M, Room 213) and in SRI's Records Center. The raw data will be retained in Building 205, Room 13, for one year after the laboratory notebook has been filled and then will be stored in SRI's Records Center. All that remains of Compound T-3752 will be kept for six months in our chemical storage room (Building M, Room 217) and then returned to 3M Company.

MATERIALS

- Test Article
  - Name: T-3752
  - Date Received: 15 May 1985
  - Description: Amber waxy solid
  - Storage Conditions: Stored at room temperature in a secondary container
  - Special Testing Conditions: None
  - Stability: Assured by Sponsor

- Indicator Organisms
  - Species: *Salmonella typhimurium* LT2; *Saccharomyces cerevisiae*
  - Strains: TA1535, TA1537, TA1538, TA98, and TA100 for *S. typhimurium*; D3 for *S. cerevisiae*
  - Source: Dr. Bruce Ames, University of California, Berkeley, for the *Salmonella*; Dr. F. K. Zimmermann, W. Germany, for the *Saccharomyces*

- Metabolic Activation

  Aroclor 1254-induced, rat liver S-9; SRI Batch F-5;
  ~ 31.5 mg/ml protein

  Animal Supplier: Simonsen Laboratories, Gilroy, California

- Negative (Solvent/Diluent) Control Material

  Dimethylsulfoxide (DMSO), CAS No. 67-68-5
  Date Opened: 3 and 8 May and 3 June 1985
  Expiration Date: 3 and 8 May and 3 June 1986, respectively
  Manufacturer: American Scientific Products, McGraw Park, IL
  Purity: 0.12%--H_2O (for all)
  Lot No.: 4948 KVLX (for all)

- Positive Control Chemicals

9-Aminoacridine, CAS No. 90-45-9
Manufacturer: Pfaltz and Bauer, Stamford, CT

2-Anthramine, CAS No. 613-13-8
Manufacturer: Sigma Chemical Co., St. Louis, MO

2-Nitrofluorene, CAS No. 607-57-8
Manufacturer: Aldrich Chemical Co., Milwaukee, WI

Sodium azide, CAS No. 26628-22-8
Manufacturer: Difco Laboratories, Detroit, MI

1,2:3,4-Diepoxybutane, CAS No. 1464-53-5
Manufacturer: Pfaltz and Bauer, Stamford, CT

Sterigmatocystin, CAS No. 10048-13-2
Manufacturer: Aldrich Chemical Co., Milwaukee, WI

- Counters Used

  - New Brunswick Scientific BioTran II® Automated Colony Counter, Model C111, SRI Nos. 0030 6126 00, 0030 0151 00, and 0012 3318 00

  - New Brunswick Scientific Bactronic® Colony Counter, Model C110, SRI Nos. 0030 1471 00, 0012 3108 00, and 0013 0788 00

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 medium 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 visible 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, usually in a dose-related manner.

We obtained our *S. typhimurium* strains from Dr. Bruce Ames of the University of California at Berkeley. 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 molecules, thereby increasing the mutagenic effect of these molecules. The *uvrB* mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically induced DNA lesions and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to *his*$^+$ by many mutagens that cause base-pair substitutions. Strain 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. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect

the presence of the plasmid in the cell. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. 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 frozen in nutrient broth supplemented with 10% sterile glycerol at -80°C in 1-ml aliquots containing about 10^9 cells. New frozen stock cultures are made every three months from single colony isolates that have been checked for their genotypic characteristics (*his*, *rfa*, *uvrB*, *bio*) and for the presence of the plasmid. For each experiment, the frozen 1-ml cell cultures are allowed to thaw at room temperature before inoculation in 50 ml of glucose minimal liquid medium supplemented with an excess of biotin and histidine. The cultures are grown at 37°C, unshaken for 4 hours, then gently shaken (100 rpm) for 11 to 14 hours. All strains are genetically analyzed whenever experiments are performed.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amine 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. 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. In brief, adult male Sprague-Dawley rats (200 to 250 g) are given a single 500-mg/kg intraperitoneal injection of Aroclor 1254 (a mixture of polychlorinated biphenyls). 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 with an equal volume of cold, sterile 0.15 M KCl, minced with sterile surgical scissors in three volumes of 0.15 M KCl (3 ml/g of wet organ), and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 × g, and the supernatant, referred to as the S-9 fraction, is quickly frozen on dry ice and stored at -80 °C.

The metabolic activation mixture for each experiment consists of, for 50 ml:

- 5.0 ml of S-9 fraction
- 1.0 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.25 ml of glucose-6-phosphate (1 M)
- 2.0 ml of NADP (0.1 M)
- 25.0 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 16.75 ml of sterile H_2O.

The amount of S-9 fraction delivered to each plate is 50 µl.

Plate Incorporation Assay

Prior to testing, the test article is serially diluted from an initial stock. Generally, a preliminary experiment is conducted to find a suitable dose range for testing. The article is usually tested over a minimum of six dose levels, the highest nontoxic dose level being 10 mg/plate unless solubility, mutagenicity, or toxicity dictates a lower upper limit. When extracts are made, various undiluted aliquots are tested, usually over a dose range of 5 to 100 or 200 µl/plate. When liquids are tested, occasionally the sample is not diluted and various aliquots are used. All assays are repeated at least once on a separate day.

The plate incorporation assay is performed in the following way. To a sterile 13 × 100-mm test tube placed in a 43 °C heating block we add:

(1) 2.00 ml of 0.6% agar containing 0.6% NaCl, 0.05 mM biotin, and 0.05 mM histidine

(2) 0.05 ml of indicator organisms (about 10^8 bacteria)

(3) 0.05 ml of a solution of the test article

(4) 0.50 ml of metabolic activation mixture (if appropriate).

This mixture is stirred gently and then poured on plates containing about 25 ml of minimal glucose agar. After the top agar has set, the plates are incubated for 48 hours at 37°C. The number of $\underline{his}^+$ revertant colonies is counted using a BioTran II automated colony counter when possible. When accurate counts cannot be obtained (e.g., because of precipitate), the plates are counted manually using an electric probe colony counter.

Concurrent sterility, negative (solvent/diluent), and positive controls are run with every experiment. Sterility controls include plating out separately steps (3) and (4). For negative controls, we use steps (1), (2), (4), and 0.05 ml of the solvent/diluent used for the test article, if appropriate. For positive controls, we test each bacterial culture using steps (1), (2), (3), and (4) with the following mutagens:

- Sodium azide for the base-pair substitution mutants TA1535 and TA100
- 9-Aminoacridine for the frameshift mutant TA1537
- 2-Nitrofluorene for the frameshift mutants TA1538 and TA98
- 2-Anthramine for all tester strains, in the presence of metabolic activation.

We routinely check for true revertant ($\underline{his}^+$) colonies by replica plating from the parent to minimal glucose agar plates supplemented with biotin.

Criteria for Interpretation

Positive. A test article is considered a mutagen when it produces a reproducible, dose-related increase in the number of revertants in one or more strains. This increase should occur for at least three dose levels.

Negative. A test article is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments. The maximum dose level tested for nontoxic

compounds is 10 mg/plate (unless dictated otherwise by the sponsor or by solubility problems). For toxic compounds, only the highest dose level tested should show evidence of toxicity.

Inconclusive. When a test article cannot be identified clearly as a mutagen or nonmutagen in the standard plate assay, the results are classified as inconclusive.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enyzme 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 carcinogenic or recombinogenic agents. The recombinogenic activity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on test plates.

A culture of S. cerevisiae is stored at 4°C. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C, with shaking.

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 10^8 cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.0 ml of the resuspended culture
- 0.5 ml of either the metabolic activation mixture or buffer
- 0.2 ml of the test chemical
- 0.3 ml of buffer.

Several doses of the test chemical are tested (up to 5% w/v or v/v) in each experiment, and appropriate solvent/diluent controls are included. 1,2:3,4-Diepoxybutane without metabolic activation and sterigmatocystin with activation are used as positive controls.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiologic saline, and 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on plates containing the same ingredients as the broth plus 2.0% agar; five plates are spread with the 10^{-3} dilution and three plates are spread with the 10^{-5} dilution. The plates are incubated for 3 days at 30°C, followed by at least 1 day at 4°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates containing the 10^{-3} dilution are scanned with a dissecting microscope at 10× 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.

Criteria for Interpretation

Positive. 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 milliliter and in the relative number of mitotic recombinants per 10^5 survivors.

Negative. When no reproducible recombinogenic activity is obtained in any of the assays performed, the test results are considered to be negative.

Inconclusive. When a test article cannot be identified clearly as causing a positive or a negative response, the results are classified as inconclusive.

Statistical Analysis

No statistical analysis is performed for any of the assays. The results of the plate incorporation assay are a tabulation of the number of colonies appearing on the plates. The results of the *S. cerevisiae* D3 assay are tabulated after calculating the number of mitotic recombinants per 10^5 survivors. All calculations are expressed with two significant digits.

References

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RESULTS AND DISCUSSION

3M Company's Compound T-3752 was screened for mutagenic activity in the Ames Salmonella/microsome in vitro mutagenicity assay using the five standard strains of Salmonella typhimurium: TA1535, TA1537, TA1538, TA98, and TA100. The assays were performed in duplicate, using three plates per dose, both in the presence and absence of a rat-liver metabolic activation system. DMSO was used as the solvent.

The microbial mutagenicity testing of this sample was performed twice at dose levels ranging from 10 to 5000 µg/plate (Tables 1 and 2). No increases in the number of revertant colonies over the spontaneous background were observed under any of the assay conditions used. Background lawn thinning was observed in the first assay only with strain TA1537 on all of the plates at 5000 µg/plate and on two of the three plates at 1000 µg/plate without activation. A light precipitate was noted at 5000 µg/plate; therefore, these plates were hand-counted. The results are presented in Tables 1 and 2.

Compound T-3752 was also screened for recombinogenic activity in the yeast Saccharomyces cerevisiae D3 assay for mitotic recombination. The assays were performed twice on separate days, both in the presence and absence of a rat-liver metabolic activation system. DMSO was used as the solvent. Since no toxicity was seen in the range-finding assay, the first experiment was performed at dose levels of 0.05, 0.1, 0.5, 1.0 and 5.0% (w/v). No increases in the number of mitotic recombinants were observed. The confirmatory assay was performed under conditions identical to those used in the initial assay. Again, no recombinogenic response was observed. The results of these two experiments are presented in Tables 3 and 4.

In conclusion, Compound T-3752 was reproducibly nonmutagenic and nonrecombinogenic when tested according to the procedures outlined in this report.

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COMPOUND T-3752

Experiment Date: 28 May 1985

Compound	Metabolic Activation	Compound Added per Plate	Histidine Revertants per Plate														
			TA1535			TA1537			TA1538			TA98			TA100*		
Negative Control DMSO	–	50 µl	19	22	27	6	7	3	22	29	15	19	26	28	117	104	108
	+	50	8	16	6	3	9	10	23	17	39	31	35	17	130	122	135
Positive Controls																	
Sodium azide	–	1 µg	372	280	386										342	321	346
9-Aminoacridine	–	50				175	165	170									
2-Nitrofluorene	–	5							1686	1329	1284	818	883	848			
2-Anthramine	–	1							18	33	28	19	31	21	148	112	135
	+	1							230	214	808	97	109	355	230	280	330
	–	2.5	28	18	17	13	5	11									
	+	2.5	158	177	61	31	33	25									
Compound T-3752	–	10 µg	17	16	24	10	3	8	24	18	17	20	14	19	148	122	143
	–	50	10	9	15	5	5	6	9	20	28	14	18	20	106	117	82
	–	100	15	14	22	5	7	6	14	15	20	13	21	27	122	137	105
	–	500	12	11	21	4	6	7	23	17	27	17	22	30	143	114	142
	–	1000	9	15	17	8†	2†	13	14	15	21	19	19	12	140	151	154
	–	5000‡	14	11	18	5†	7†	8†	15	24	15	21	17	16	106	89	93
	+	10	13	7	17	13	8	5	26	34	33	41	30	32	98	121	101
	+	50	12	8	3	13	12	7	22	30	31	39	33	28	87	117	138
	+	100	14	5	13	6	5	6	35	18	36	31	27	34	109	78	85
	+	500	10	3	7	4	9	6	14	31	18	36	33	29	125	126	86
	+	1000	13	13	8	9	11	8	28	23	22	32	24	29	126	132	96
	+	5000‡	8	6	9	7†	5†	9†	28	35	27	43	39	50	93	97	124

*Experiment performed on 14 June 1985.
†Background lawn thinning.
‡Precipitated at this dose level; hand-counted.

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COMPOUND T-3752

Experiment Date: 6 June 1985

Compound	Metabolic Activation	Compound Added per Plate	Histidine Revertants per Plate														
			TA1535			TA1537			TA1538*			TA98*			TA100		
Negative Control DMSO	-	50 µl	31	37	27	3	10	3	16	15	18	20	17	23	160	129	123
	+	50	18	14	13	9	9	8	30	24	23	38	24	18	136	114	122
Positive Controls																	
Sodium azide	-	1 µg	562	586	621										600	666	636
9-Aminoacridine	-	50				456	307	390									
2-Nitrofluorene	-	5							1111	1017	842	675	452	502			
2-Anthramine	-	1							24	18	20	23	17	34	173	174	127
	+	1							202	160	256	109	108	194	306	336	311
	-	2.5	33	30	38	13	13	12									
	+	2.5	214	182	177	51	59	49									
Compound T-3752	-	10 µg	42	24	34	9	10	12	21	17	11	15	24	13	117	137	136
	-	50	32	33	36	3	10	11	14	17	17	24	30	20	153	179	127
	-	100	33	27	45	13	9	4	15	13	8	28	38	26	148	140	150
	-	500	35	42	33	6	9	8	19	11	17	23	18	14	140	152	130
	-	1000	31	33	31	6	8	10	7	13	12	10	25	14	143	126	122
	-	5000†	39	24	31	11	7	8	12	16	15	12	14	17	133	103	130
	+	10	16	11	10	13	7	12	18	16	19	25	20	29	174	108	136
	+	50	10	13	17	9	7	13	30	17	32	22	18	29	140	108	136
	+	100	10	11	20	12	3	17	23	24	36	22	24	23	153	128	146
	+	500	17	16	12	9	7	6	31	32	17	24	27	20	147	138	121
	+	1000	12	15	9	10	12	11	22	22	18	38	29	20	130	128	147
	+	5000†	18	18	18	15	11	18	37	35	33	28	17	28	150	101	130

*Experiment performed on 14 June 1985.
†Precipitated at this dose level; hand-counted.

Table 3

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

COMPOUND T-3752

Experiment Date: 24 May 1985

Compound	Metabolic Activation	Percent Concentration (w/v)	Surviving Cells per ml ($\times 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($\times 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors*
Negative Control						
DMSO	-		7.3	100	4.5	6.2
	+		7.2	100	7.5	10
Positive Controls						
1,2:3,4-Diepoxy-butane	-	0.025	7.1	97	1331	1900
Sterigmatocystin	-	0.005	6.8	93	2	2.9
	+	0.005	7.4	100	303	410
Compound T-3752	-	0.05	7.2	99	9	13
	-	0.1	7.1	97	6	8.5
	-	0.5	6.9	95	7	10
	-	1.0	7.1	97	11	15
	-	5.0	7.6	100	5	6.6
	+	0.05	7.8	100	7	9.0
	+	0.1	6.4	89	9	14
	+	0.5	7.2	100	8	11
	+	1.0	6.0	83	6	10
	+	5.0	8.0	100	7	8.8

*All calculations are expressed to two significant figures.

Table 4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

COMPOUND T-3752

Experiment Date: 7 June 1985

Compound	Metabolic Activation	Percent Concentration (w/v)	Surviving Cells per ml ($\times 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($\times 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors*
Negative Control						
DMSO	-		7.3	100	8	11
	+		6.9	100	10	14
Positive Controls						
1,2:3,4-Diepoxy-butane	-	0.025	7.3	100	1512	2100
Sterigmatocystin	-	0.005	7.3	100	8	11
	+	0.005	7.3	100	434	590
Compound T-3752	-	0.05	7.5	100	8	11
	-	0.1	7.0	96	9	13
	-	0.5	6.9	95	13	19
	-	1.0	6.9	95	6	8.7
	-	5.0	7.5	100	6	8.0
	+	0.05	7.2	100	6	8.3
	+	0.1	7.6	100	7	9.2
	+	0.5	7.1	100	9	13
	+	1.0	7.3	100	11	15
	+	5.0	7.7	100	11	14

*All calculations are expressed to two significant figures.