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IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY COMPOUNDS T-2540 CoC and T-2541 CoC

Final Report

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SUMMARY

SRI International examined 3M Company compounds T-2540 CoC and T-2541 CoC for mutagenic activity with strains TA1535, TA1537, TA1538, TA98, and TA100 of Salmonella typhimurium in the standard Ames Salmonella/microsome assay, in an assay conducted in desiccators, 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. Neither T-2540 CoC nor T-2541 Coc was mutagenic or recombinogenic in any of the assays performed.

INTRODUCTION

SRI International examined 3M Company compounds T-2540 CoC and T-2541 CoC 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, in an assay conducted in desiccators, 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.[2,3] 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 medium agar plates containing a trace of histidine, only 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. 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.[1,4-7] 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 causes decreased 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.[7] 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.[8] The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICI-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 at 4°C on minimal agar plates supplemented with an excess of biotin and histidine. The plates with the plasmid-carrying strains also contain ampicillin (25 µg/ml) to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every four to six weeks 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, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid, CM67).

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.[6,9-11] 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.[9] 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 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 (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized 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 $MgCl_2$ (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 H_2O.

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43°C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (optional)
(4) 0.05 ml of a solution of the test chemical.

This mixture is stirred gently and then poured onto minimal agar plates.† After the top agar has set, the plates are incubated at 37°C for 3 days. The number of *his*$^+$ revertant colonies is counted and recorded.

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*The 0.6% agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.6% NaCl.

†Minimal agar plates consist of, per liter, 15 g of agar, 10 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaHNH_4PO_4 \cdot 4H_2O$.

For negative controls, we use steps (1), (2), and (3) (optional), and 0.05 ml of the solvent is used for the test chemical. Dimethylsulfoxide (DMSO) was used as the solvent for T-2540 CoC and T-2541 CoC. For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3), (optional), and (4).

Assays in Desiccators for Volatile Compounds

The standard Ames plate test is not entirely suitable for testing highly volatile chemicals, so we have modified the procedure to conduct such testing. The Salmonella plates are prepared as described for the assays in agar, but no test chemical is added. The plates, with lids removed, are placed side by side on a perforated shelf in a 9-liter desiccator (Figure 1). A known volume of the test chemical is added to a glass petri plate that is placed in the center of and attached to the bottom of the shelf. A control chemical is tested similarly in each experiment. The desiccator is sealed and placed on a magnetic stir plate in a room maintained at 37°C. A magnetic stirrer with vanes, placed in the base of each desiccator, ensures adequate dispersion of the chemical. After incubation for 8 hours, the plates are removed from the desiccators, their lids are replaced, and they are incubated at 37°C for an additional 64 hours. The number of his^+ revertants is counted and recorded.

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.[2] 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.[3]

DESICCATOR ASSAY

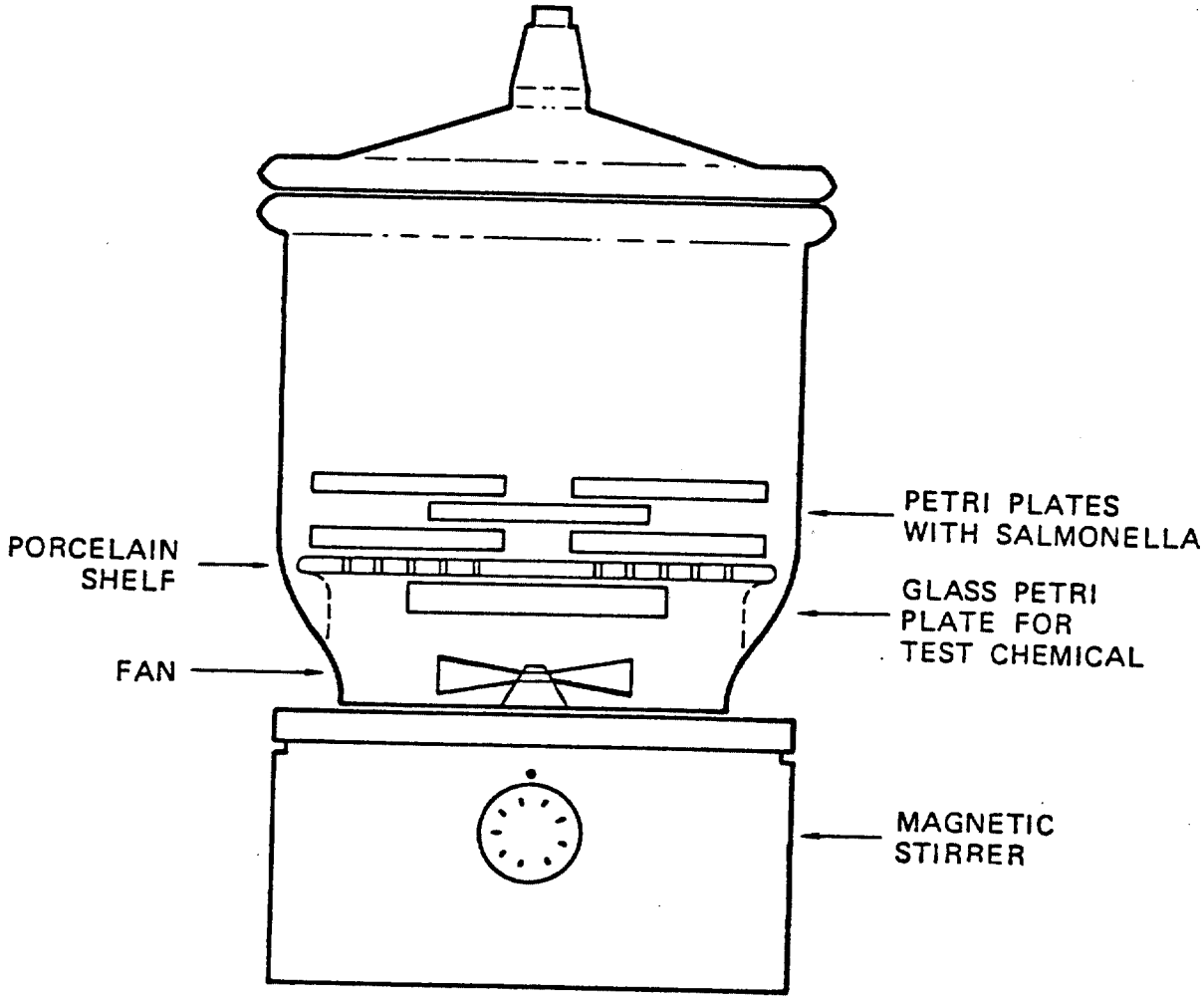

Figure 1

A stock 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:

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

DMSO was used as the solvent for T-2540 CoC and T-2541 CoC. Several dose levels 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 physiologic 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 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 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 containing 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 3-fold in the absolute number of mitotic recombinants per milliliter 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 our tests of T-2540 CoC and T-2541 CoC in the Ames Salmonella/microsome assay. The data in each table show the results of a duplicate assay performed on separate days. Both compounds were tested over a wide range of dose levels, from 10 to 5,000 µg/plate, both with and without metabolic activation. Compound T-2540 CoC was toxic to the bacteria at 5,000 µg/plate. No dose-related increase in the number of histidine revertants over the background count was observed in either assay. Therefore, we conclude that compounds T-2540 CoC and T-2541 CoC were not mutagenic in the standard Salmonella plate incorporation assay.

Table 3 presents the results of an assay of T-2540 CoC and T-2541 CoC conducted in desiccators with strains TA98 and TA100. The compounds were tested over a range of dose levels from 0.1 to 5.0 ml per desiccator. Toxicity was observed at 1.0 ml per desiccator with T-2540 CoC and at 5.0 ml per desiccator with T-2541 CoC. Because no mutagenic response was observed with or without metabolic activation, no further testing was performed in desiccators.

The results of microbiological assays with S. cerevisiae D3 on T-2540 CoC are presented in Tables 4 through 6. In a preliminary experiment conducted over a range of concentrations from 0.1 to 5.0% (Table 4), organisms exposed to T-2540 CoC showed less than 50% survival at concentrations of 0.5% and higher. Therefore, the compound was retested over a range of concentrations from 0.025 to 0.25% (Table 5). There was a slight increase in the number of mitotic recombinants both with and without metabolic activation at concentrations from 0.075 to 0.25%, and this increase was not dose-related. Compound T-2540 CoC was tested once more within a narrower range, from 0.07 to 0.2% without activation and from 0.09 to 0.4% with activation (Table 6). In this assay, there was an increase in the number of mitotic recombinants only

at 0.3% with activation. Because the increases in mitotic recombinants observed in both assays were neither dose-related nor reproducible, we do not believe that T-2540 CoC was recombinogenic with S. cerevisiae D3.

Tables 7 through 9 present the results of assays with T-2541 CoC with S. cerevisiae D3. A preliminary experiment determined that T-2541 CoC was toxic to the yeast at 0.5% without activation and 5.0% with activation (Table 7). The compound was tested twice more at concentrations from 0.025 to 0.25% without activation and 0.25 to 2.5% with activation (Table 8), and at concentrations from 0.07 to 0.2% without activation and 0.2 to 1.0% with activation (Table 9). T-2541 CoC showed several increases in the number of mitotic recombinants per 10^5 survivors. However, as with T-2540 CoC, these increases were neither dose-related nor repeatable; therefore, we conclude that T-2541 CoC was not recombinogenic in this assay.

In summary, we conclude that compound T-2540 CoC and T-2541 CoC were not mutagenic with S. typhimurium, or recombinogenic with S. cerevisiae D3.

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative control (DMSO)	-		27	24	9	12	28	18	31	43	125	112
	+		28	28	43	48	50	41	53	43	110	136
Positive control												
Sodium azide	-	1.0	569	531							836	859
9-Aminoacridine	-	50.0			576	261						
2-Nitrofluorene	-	5.0					727	537	371	387		
2-Anthramine	-	1.0					17	20	20	31	136	102
	+	1.0					251	306	268	219	373	417
	-	2.5	21	26	18	7						
	+	2.5	182	194	81	67						
T-2540 CoC	-	10	26	19	12	8	18	20	40	21	124	103
	-	50	19	37	7	13	16	15	25	37	98	88
	-	100	19	24	8	7	14	14	27	27	101	102
	-	500	16	27	4	8	16	19	26	39	117	100
	-	1,000	32	18	19	9	18	17	32	42	111	100
	-	5,000	19T*	19T	6T	5T	15T	12T	16T	30	85T	86T
	+	10	33	25	36	29	28	28	51	42	105	99
	+	50	31	37	33	39	24	29	42	48	108	115
	+	100	24	29	40	26	26	32	33	48	134	126
	+	500	30	31	18	30	26	33	33	39	100	125
	+	1,000	21	39	30	27	36	30	39	29	97	126
	+	5,000	26	28	18	19	38T	25T	44	43	104T	93T

(Continued)

Table 1 (Concluded)

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
T-2541 CoC	-	10	27	31	12	7	26	17	24	26	113	105
	-	50	20	28	4	5	15	15	43	25	115	127
	-	100	33	29	6	6	12	6	30	38	90	100
	-	500	19	28	17	6	12	9	32	27	123	97
	-	1,000†	32	25	17	20	12	21	33	31	105	102
	-	5,000†	29	25	13	13	15	17	33	27	109	112
	+	10	25	18	21	30	29	17	38	50	98	110
	+	50	29	17	19	36	32	25	44	38	114	109
	+	100	28	27	28	26	19	29	45	43	126	103
	+	500	20	28	30	30	15	21	48	44	142	135
	+	1,000†	38	29	32	26	37	30	32	50	135	112
	+	5,000†	30	46	27	24	26	30	51	47	109	111

*T, toxic.

†The compound formed a precipitate at this concentration.

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control	-		21	20	5	10	16	15	17	19	109	102
DMSO	+		7	21	28	9	13	28	32	26	105	103
Positive Control												
Sodium azide	-	1.0	505	380								
9-Aminoacridine	-	50.0			442	390					624	667
2-Nitrofluorene	-	5.0					708	678	383	386		
2-Anthramine	-	1.0					21	20	25	25	116	101
	+	1.0					420	258	126	141	576	430
	-	2.5	19	24	5	6						
	+	2.5	221	256	131	158						
T-2540 CoC	-	10	20	38	6	8	9	17	26	32	90	125
	-	50	25	28	7	14	19	14	25	20	92	87
	-	100	33	17	7	14	16	20	20	16	109	92
	-	500	29	20	12	3	8	9	19	28	93	101
	-	1,000	21	16	12	5	13	7	24	27	88	108
	-	5,000	T*	6T	T	1T	9T	5T	14T	20T	67T	67T
	+	10	17	13	21	13	15	17	31	27	96	88
	+	50	19	16	22	14	14	15	29	29	98	108
	+	100	10	19	25	15	17	18	26	43	89	98
	+	500	16	4	14	12	7	20	27	31	76	97
	+	1,000	18	5	19	15	14	23	28	29	93	99
	+	5,000	14	15	14	14	18	16	14	21	81	33T

(Continued)

Table 2 (Concluded)

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
T-2541 CoC	-	10	29	21	8	8	9	13	28	17	109	96
	-	50	26	28	4	7	6	8	13	24	108	99
	-	100	34	24	13	7	13	9	29	26	101	113
	-	500	21	28	8	9	8	8	19	27	90	101
	-	1,000†	20	26	13	13	13	7	17	21	99	111
	-	5,000†	15	19	9	14	12	7	26	20	104	103
	+	10	7	15	13	9	26	17	20	32	85	96
	+	50	12	15	11	7	17	15	24	25	92	97
	+	100	18	15	8	6	25	21	24	18	101	87
	+	500	9	8	14	8	12	24	27	29	101	91
	+	1,000†	14	14	18	27	24	17	32	19	98	109
	+	5,000†	13	14	12	18	19	24	19	31	110	103

*T, toxic

†The compound formed a precipitate at this concentration.

Table 3

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS
8-HOUR EXPOSURE
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Milliliters of Compound in Desiccator	Histidine Revertants per Plate			
			TA98		TA100	
Negative Control DMSO	-		16	30	160	131
	+		33	51	142	161
Positive Control Methylene chloride	-	1.0	757	847	1478	1725
	+	1.0	817	807	1513	1476
T-2540 CoC	-	0.1	26	29	138	152
	-	0.5	28	14	99	110
	-	1.0	12T*	25T	T	5T
	-	5.0	T	T	T	T
	+	0.1	28	38	123	135
	+	0.5	52	52	139	158
	+	1.0	16T	9T	17T	15T
	+	5.0	T	T	T	T
T-2541 CoC	-	0.1	37	27	125	140
	-	0.5	24	28	97	128
	-	1.0	21	29	109	141
	-	5.0	19	13T	16T	51T
	+	0.1	36	46	138	111
	+	0.5	41	31	149	148
	+	1.0	28	29	124	127
	+	5.0	29	29	99T	103T

*T, toxic.

Table 4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2540 CoC

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	-		6.5	100	3.5	5.4
DMSO	+		7.2	100	3.5	4.9
Positive Control	-	0.025	2.5	38	608	2400
1,2,3,4 Diepoxybutane	+	0.025	5.8	81	928	1600
T-2540 CoC	-	0.1	5.6	86	5.0	8.9
	-	0.5	2.3	35	2.0	8.7
	-	1.0	1.5	23	3.0	20
	-	5.0	T*	T	T	T
	+	0.1	7.7	107	6.0	7.8
	+	0.5	3.5	49	4.0	11
	+	1.0	1.1	15	3.0	27
	+	5.0	T	T	T	T

*T, toxic.

Table 5

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2540 CoC

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	-		7.2	100	2.0	2.8
DMSO	+		7.6	100	2.5	3.3
Positive Control	-	0.025	3.6	50	923	2600
1,2,3,4 Diepoxybutane	+	0.025	7.0	92	1040	1500
T-2540 CoC	-	0.025	6.8	94	4.0	5.9
	-	0.05	6.5	90	7.0	11
	-	0.075	5.8	81	11.0	19
	-	0.1	5.7	79	6.0	11
	-	0.25	2.7	38	10.0	37
	+	0.025	7.4	97	8.0	11
	+	0.05	7.0	92	3.0	4.3
	+	0.075	5.2	68	8.0	15
	+	0.1	5.1	67	6.0	12
	+	0.25	5.3	70	14.0	26

Table 6

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2540 CoC

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	-		8.3	100	3.0	3.6
DMSO	+		7.6	100	4.0	5.3
Positive Control	-	0.025	5.2	63	880	1700
1,2,3,4 Diepoxybutane	+	0.025	6.3	83	680	1100
T-2540 CoC	-	0.07	7.8	94	6.0	7.7
	-	0.08	8.2	99	3.0	3.7
	-	0.09	6.0	72	5.0	8.3
	-	0.1	5.7	69	3.0	5.3
	-	0.2	4.7	57	7.0	15
	+	0.09	9.3	122	5.0	5.4
	+	0.1	6.1	80	5.0	8.2
	+	0.2	5.8	76	4.0	6.9
	+	0.3	3.2	42	10	31
	+	0.4	3.0	39	3.0	10

Table 7

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2541 CoC

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	-		6.5	100	3.5	5.4
DMSO	+		7.2	100	3.5	4.9
Positive Control	-	0.025	2.5	38	608	2400
1,2,3,4 Diepoxybutane	+	0.025	5.8	81	928	1600
T-2541 CoC	-	0.1	5.0	77	1.0	2.0
	-	0.5	2.3	35	4.0	17
	-	1.0	T*	T	T	T
	-	5.0	T	T	T	T
	+	0.1	7.0	97	4.0	5.7
	+	0.5	5.6	78	2.0	3.6
	+	1.0	5.1	71	4.0	7.8
	+	5.0	2.1	29	6.0	29

*T, toxic.

Table 8

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2541 CoC

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	-		7.2	100	2.0	2.8
DMSO	+		7.6	100	2.5	3.3
Positive Control	-	0.025	3.6	50	923	2600
1,2,3,4 Diepoxybutane	+	0.025	7.0	92	1040	1500
T-2541 CoC	-	0.025	5.5	76	6.0	11
	-	0.05	5.5	76	3.0	5.5
	-	0.075	5.1	71	3.0	5.9
	-	0.1	5.7	79	5.0	8.8
	-	0.25	2.7	38	7.0	26
	+	0.25	5.5	72	8.0	15
	+	0.5	5.1	67	7.0	14
	+	0.75	3.6	47	2.0	5.6
	+	1.0	3.1	41	7.0	23
	+	2.5	T*	T	T	T

*T, toxic.

Table 9

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2541 CoC

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	-		8.3	100	3.0	3.6
DMSO	+		7.6	100	4.0	5.3
Positive Control	-	0.025	5.2	63	880	1700
1,2,3,4-Diepoxybutane	+	0.025	6.3	83	680	1100
T-2541 CoC	-	0.07	8.2	99	5.0	6.1
	-	0.08	7.7	93	3.0	3.9
	-	0.09	6.0	72	6.0	10
	-	0.1	6.4	77	8.0	13
	-	0.2	5.2	63	6.0	12
	+	0.2	6.5	86	4.0	6.2
	+	0.4	8.7	114	5.0	5.8
	+	0.6	6.2	82	6.0	9.7
	+	0.8	5.4	71	11	20
	+	1.0	4.4	58	5.0	11

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