TRADE SECRET

Study Title

H-24119: Bacterial Reverse Mutation Test
in *Salmonella typhimurium* and *Escherichia coli*

Laboratory Project ID: DuPont-3220

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.5100 (1998)

OECD Guidelines for Testing of Chemicals
Section 4: Health Effects, No. 471 (1997)

Commission Directive 92/69/EEC
EEC Methods B.13 and B.14

AUTHOR: N. Lawrence Gladnick, B.A.

STUDY COMPLETED ON: July 23, 1999

PERFORMING LABORATORY: E. I. du Pont de Nemours and Company
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WORK REQUEST NO.: [redacted]

SERVICE CODE NO.: [redacted]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) and/or EPA TSCA (40 CFR 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (OCDE/GD(92)32) except for the items documented below. These items did not impact the validity of the study.

1. Treatment solutions were not analyzed for identity, composition, uniformity, or stability of the test and control substances. The procedures used by trained personnel to prepare the treatment solutions were intended to ensure:

   - The accuracy of concentration because the test and control substances were weighed on an analytical balance accurate to 3 decimal places and the dimethyl sulfoxide and water in which the test and control substances were dissolved were accurately measured with graduated pipettes or flasks;

   - Uniformity because all treatment solutions were mixed prior to administration to the test system; and

   - Stability because treatment solutions were prepared just prior to administration to the test system.

2. The test substance was characterized by the sponsor prior to initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

3. The preparation pages for ICR-191 and NAAZ, positive controls used in the study, could not be located as of the date of report issue. Quality Assurance personnel verified the existence of the prepared test materials, and the positive control data met acceptability criteria set forth in the general testing SOP. This does not impact the study integrity or validity.

Submitter/Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: N. Larry Gladnick 23 JUL 1999
N. Lawrence Gladnick, Date
B.A. Toxicology Associate

CERTIFICATION

I, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Issued by Study Director: N. Larry Gladnick 23 JUL 1999

N. Lawrence Gladnick, B.A.
Toxicology Associate

Date

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STUDY INFORMATION

9th Collective Nomenclature: Poly(difluoromethylene),.alpha.-fluoro-.omega.-[2-[(2-methyl-1-oxo-2-propenyl)oxy]ethyl]-, polymer with 2-Propenoic acid, 2-methyl-, ammonium salt and 2-propenoic acid, 2-methyl-, 2-(diethylamino)ethyl ester

Synonyms/Codes:
- H-24119
- [redacted]

Haskell Number: 24119

CAS Registry Number: [redacted]

Submitter's Notebook Number(s): E88438-149

Purity: 95%

Known Impurities:
- [redacted]

Physical Characteristics: Opaque, light yellow emulsion (20% solids)

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: June 8, 1999 / (see report cover page)

In-Life Initiated/Completed: June 9, 1999 / June 25, 1999

STUDY PERSONNEL

Study Director: N. Lawrence Gladnick, B.A.
Management: David F. Krahn, Ph.D. / Ronald D. Snyder, Ph.D.
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SUMMARY

H-24119 was evaluated in the bacterial reverse mutation assay using *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, and *Escherichia coli* strain WP2 *uvrA* (pKM101) in the presence and absence of an exogenous metabolic activation system (Aroclor®-induced rat liver S9). The single trial assay was performed using the plate incorporation method to evaluate the mutagenic potential of the test substance. All tester strains exhibited appropriate phenotypic characteristics. For all tester strains the mean number of revertants scored for the negative controls was within the prescribed acceptable range derived from historical data.

Concentrations of 5, 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate were assessed in comparison to negative (solvent) controls. The solvent control and diluent used in this study was sterile water. No toxicity was observed as evidenced through the reduction of the microcolony background lawns or as a concentration-related reduction in the mean number of revertants per plate. No precipitate was observed at any concentration in any strain.

Under the conditions of this study, no evidence of mutagenic activity was detected in any of the *Salmonella* strains or the *Escherichia coli* strain. The test substance was negative.

INTRODUCTION

This study evaluated the mutagenic potential of the test substance, H-24119, in *Salmonella typhimurium* strains TA97a, TA98, TA100, and TA1535 and in *Escherichia coli* strain WP2 *uvrA* (pKM101). The bacterial reverse mutation test uses amino acid requiring strains of *Salmonella typhimurium* and *Escherichia coli* to detect mutations, that involve substitution, addition or deletion of 1 or a few DNA base pairs.[1,2] The *Salmonella* tester strains are unable to synthesize histidine because of specific point mutations in genes coding for histidine biosynthesis. Additional mutation of the defective gene specific to the tester strain can result in individual bacteria regaining the ability to synthesize histidine. Tester strains TA97a and TA98 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) primarily by frameshift mutagens. Tester strains TA100 and TA1535 are reverted by mutagens that primarily cause base pair substitutions. *E. coli* WP2 *uvrA* (pKM101) is unable to synthesize tryptophan due to an ochre mutation in the gene required for tryptophan biosynthesis. *E. coli* WP2 *uvrA* (pKM101) is primarily sensitive to mutagens that act at AT base pairs within the *trpE* gene and may also revert to prototrophy from suppressor mutations at a locus in a tRNA gene.[2] By comparing the number of chemically induced revertants to the number of spontaneous revertants, the mutagenicity of the test substance can be assessed.

MATERIALS AND METHODS

A. STUDY PROTOCOL

The protocol consisted of the Short-Term Study Protocol Sheet and the Haskell General Testing Procedure GT008-T-010 ("Bacterial Reverse Mutation Test for Solids, Liquids, and Gases", effective 6/10/98). The study was designed to comply with:

- U.S. EPA, Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guidelines (Subpart H, 40 CFR Part 870.5100 [1998]);
- Guidelines of the Organisation for Economic Cooperation and Development (OECD Guidelines for Testing of Chemicals, No. 471 [1997]);
- The guidelines of the European Union (EEC Commission Directive 92/69/EEC, Methods B.13 and B.14 [1992]);

The study design complied with the testing guidelines cited above with the following exceptions:

- *S. typhimurium* tester strain TA97a using ICR 191 as a positive control was used as a substitute for strain(s) TA97 and/or TA1537 (EEC and Japan).
- *E. coli* WP2 *uvrA* (pKM101) was the only *E. coli* tester strain used (EEC).
- One trial was conducted with the test substance as specified by the sponsor (EEC).

These exceptions did not affect the study validity for the following reasons:

- According to some testing guidelines *S. typhimurium* strain TA1537, *Salmonella* strains TA1537, TA97 or TA97a may be used interchangeably.[3] *Salmonella* strain TA97 has been demonstrated to be more sensitive to frameshift mutagens and the reconstructed strain, TA97a, was used due to its improved growth properties.[4]

- Although some testing guidelines require *E. coli* WP2 *uvrA*, no significant difference in mutagen detection would be expected between the plasmid and non-plasmid containing strains; therefore, *E. coli* WP2 *uvrA* (pKM101) was used to assess potential mutagenicity. The strains used in this study comply with harmonized guidelines designed to minimize variations among testing procedures worldwide (OECD, OPPTS).

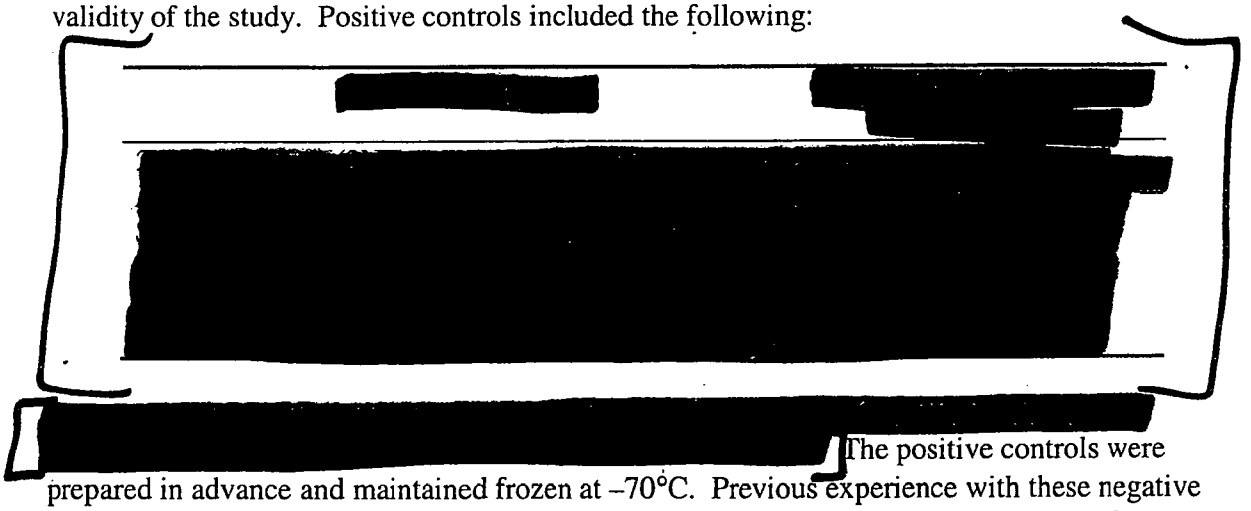
B. TEST MATERIALS

1. Test Substance

The test substance H-24119 is an opaque liquid that was stored at room temperature. Additional information regarding the test substance can be found on the study information page of this report. The test substance was assumed to be stable during the study and no evidence of instability was observed.

2. Negative and Positive Controls

Based on information supplied by the sponsor and a solubility assessment at the testing facility, sterile water was chosen as the test substance solvent, diluent, and negative control. There were no impurities, known or reasonably anticipated, in the controls that might interfere with the validity of the study. Positive controls included the following:



The positive controls were prepared in advance and maintained frozen at –70°C. Previous experience with these negative and positive controls indicates that they are stable in this test system and no evidence of instability was observed during the study.

3. Tester Strain Source, Characterization, Storage, and Culture

S. typhimurium tester strains were obtained from Dr. Bruce Ames, Berkeley, CA, USA. *E. coli* WP2 *uvrA* (pKM101) was obtained from the National Collection of Industrial Bacteria, Torrey Research Station, Scotland, UK. The characteristics of *S. typhimurium* tester strains TA97a, TA98, TA100, TA102, TA1535 and *E. coli* WP2 *uvrA* (pKM101) are as follows:

Strain	Gene Locus		LPS	R-factor (pKM101) Plasmid	pAQ1 Plasmid
	Reversion Target	DNA Repair*			
S. typhimurium TA97a	*his*D6610, *his*O1242	*uvrB*	*rfa*	Present	Absent
S. typhimurium TA98	*his*D3052	*uvrB*	*rfa*	Present	Absent
S. typhimurium TA100	*his*G46	*uvrB*	*rfa*	Present	Absent
S. typhimurium TA102	*his*G428	(+)	*rfa*	Present	Present
S. typhimurium TA1535	*his*G46	*uvrB*	*rfa*	Absent	Absent
E. coli WP2 *uvrA* (pKM101)	*trpE*	*uvrA*	NA	Present	Absent

LPS=lipopolysaccharide; (+) proficient for excision repair; NA=Not applicable
*Defective DNA repair gene.

The deletion (Δ) in *uvrB* (a gene that codes for 1 protein involved in DNA excision repair) increases the bacterial sensitivity to some mutagens.[5] The *uvrB* and *uvrA* traits are confirmed by demonstrating an increased bacterial sensitivity to ultraviolet light. Because the *uvrB* deletion also extends through a gene needed for biotin biosynthesis, the *S. typhimurium* tester strains require exogenous biotin to be added to culture media or plates for growth. The *rfa* mutation causes a partial loss in the integrity of the lipopolysaccharide (LPS) cell wall so that permeability to large molecules is increased.[6] The presence of the pKM101 or R-factor plasmid, conferring ampicillin resistance, also enhances an error-prone DNA repair system that is endogenous to these bacteria.[7] The pAQ1 plasmid confers tetracycline resistance to *S. typhimurium* TA102, that was used solely as a control for UV light sensitivity.

Salmonella tester strains were stored at -70°C in ~8% (v/v) DMSO in Oxoid Nutrient Broth No. 2. The *E. coli* WP2 *uvrA* (pKM101) strain was stored at -70°C in ~30% glycerol in Oxoid Nutrient Broth No. 2. Overnight cultures were prepared by inoculating 20 mL of Oxoid Nutrient Broth No. 2 with 0.1 mL of a bacterial stock and incubating at 37°C with shaking. Concurrent with the reverse mutation test, tester strain phenotypes were confirmed on overnight cultures.

4. Metabolic Activation System

Because the tester strains lack many of the enzymes required to convert some promutagens to a reactive state, the assay was performed in the presence and absence of an exogenous metabolic activation system similar to that described by Maron and Ames.[1] The exogenous metabolic activation system was a cofactor-supplemented post-mitochondrial fraction, (i.e., 9000 x g; homogenate of 1 g wet liver weight in 3 mL of an approximately 0.15 M KCl solution) prepared from the livers of young male Sprague Dawley rats treated with the enzyme-inducing agent Aroclor® 1254 (500 mg/kg i.p.) as a single dose 5 days prior to sacrifice. The Aroclor®-induced rat liver S9 (purchased from MOLTOX™) was characterized for protein content and metabolic activity by the vendor. To confirm the sterility of the exogenous metabolic activation system, an

aliquot was plated on nutrient agar capable of supporting the growth of viable bacteria. The amount of Aroclor®-1254 induced rat liver S9 in the exogenous metabolic activation system was 4.0 mg S9 protein (~11% [v/v]) / mL. The cofactor-supplement concentrations in the exogenous metabolic activation system were 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate (as a sodium salt), 4 mM $NADP^+$ (as a sodium salt), and 100 mM sodium phosphate buffer pH 7.4.

C. CONCENTRATION SELECTION, TEST SUBSTANCE STABILITY AND VERIFICATION

In accordance with testing guidelines, the highest concentration evaluated in this study was 5000 µg/plate. Solubility information was confirmed prior to study start.

Solutions of the test substance were prepared immediately prior to treatment and were presumed to be stable under the conditions of the study. Treatment, control solutions, and the S9 mixture were not analyzed for concentration, uniformity, or stability. Top agar was not assayed for stability or concentration of the test or control substances, strain, or S9/PBS, since this assessment was not considered necessary to achieve the objectives of the study. Solutions of the test substance were assessed for sterility by plating a small amount of the highest test substance concentration onto the surface of minimal glucose agar plates.

D. TEST METHODS: BACTERIAL MUTAGENICITY ASSAY

This study consisted of a single trial that assessed test substance mutagenicity. Three replicates were plated for each tester strain in the presence and absence of the exogenous metabolic activation system at each test substance concentration. Positive and negative controls were included for each strain and condition. Treatments with the exogenous metabolic activation system were conducted by adding 0.1 mL of negative or positive control or test substance solution, 0.5 mL of metabolic activation system, and 0.1 mL of an overnight culture containing approximately 1 x 10^8 bacteria to approximately 2 mL of top agar (0.6% [w/v] agar and NaCl) containing 0.05 mM L-histidine, D-biotin and L-tryptophan. These components were briefly mixed and poured onto a minimal glucose agar plate (25-30 mL, 0.4% [w/v] glucose with Davis salts, purchased from MOLTOX™). Treatments in the absence of the metabolic activation system were the same as those in the presence of the exogenous metabolic activation system with the exception that 0.5 mL of sterile buffer was used as a replacement for the volume of the exogenous metabolic activation system. After pouring onto the surface of minimal glucose agar plates, the top agar was allowed time to solidify, and the individually labeled plates were inverted and incubated at 37°C for about 48 hours. When necessary, plates were refrigerated at approximately 4°C (± 3°C) prior to evaluation and counting of revertant colonies.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Evidence of toxicity was scored relative to the concurrent negative control plates and recorded with the mean revertant count for the strain, condition, and concentration. Revertant colonies for a given tester strain and condition were counted by an automated colony counter.

E. STATISTICAL ANALYSIS

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of the exogenous metabolic activation system were calculated.

F. ACCEPTABILITY CRITERIA

An individual trial must have included a negative and positive control and at least 5 concentration levels of the test substance for each tester strain and condition. A data point, concentration level or trial was excluded from analysis when acceptability criteria were not met. The acceptability criteria were as follows:

1. Tester Strain Phenotypes and Spontaneous Reversion

All *S. typhimurium* tester strain cultures must have exhibited L-histidine dependent growth. To demonstrate the presence of the *rfa* mutation, all *S. typhimurium* tester strain cultures must have exhibited sensitivity to crystal violet. To demonstrate the presence of the *uvrB* mutation, all *S. typhimurium* tester strain cultures must have exhibited sensitivity to ultraviolet light in comparison to strain TA102, which is proficient in the repair of small amounts of ultraviolet light-induced DNA damage. *E. coli* tester strains must have exhibited L-tryptophan dependent growth. To demonstrate the presence of the *uvrA* mutation, the *E. coli* tester strain must have exhibited sensitivity to ultraviolet light. Tester strain cultures of *S. typhimurium* TA97a, TA98, TA100 and *E. coli* WP2 *uvrA* (pKM101) must have exhibited resistance to ampicillin.

All tester strain cultures must have exhibited a characteristic number of spontaneous revertants per plate in the absence of the test substance. The acceptable mean revertants per plate in the presence or absence of the exogenous metabolic activation system were derived from the means of the historical negative control data and were within the following ranges: *S. typhimurium* strains TA97a (65-163); TA98 (8-40); TA100 (58-192); TA1535 (2-28), *E. coli* strain WP2 *uvrA* (pKM101) (90-227). Historical data collected at the testing facility are presented in Table 6.

2. Tester Strain Culture Titer

To ensure that appropriate numbers of bacteria were plated, all tester strain culture titers were approximately 1 x 10^9 cells/mL.

3. Positive Control Values

Mean positive control values must have exhibited at least a three-fold increase over the respective mean of the concurrent negative control value for each tester strain and condition.

4. Non-Toxic Concentration Levels

A minimum of 5 analyzable, of which four must be non-toxic, concentration levels was required to classify the test substance. A concentration level was considered toxic and analyzable if it caused a >50% reduction in the mean number of revertants per plate relative to the mean of the concurrent negative control and is not equal to 0.

5. Rejection of Plates, Concentration Levels, or Assays

A plate may have been rejected if contamination, test substance precipitation or conditions resulted on a treatment plate that prevented an accurate colony counting.

A concentration level (or a negative control) was rejected if there were less than 2 data points or if variability between replicate plates was judged to be excessive. Variability between plates is strain dependent and can be effected by treatment with the test substance; therefore, scientific judgement was used in determining the acceptability of the data.

An assay (for an individual strain) was rejected if the negative control was rejected, if the positive control was rejected, or if the tester strain failed to exhibit the appropriate phenotype.

All data from the single trial were retained, and data that met acceptability criteria are included in this report.

G. CLASSIFICATION GUIDELINES

A test substance was classified as **POSITIVE** (i.e., mutagenic) if the mean number of revertants in any strain at any test substance concentration was at least 2 times greater than the mean of the concurrent vehicle control and there was a concentration-related increase in the mean revertants per plate in that same strain.

A test substance was classified as **NEGATIVE** (i.e., not mutagenic) if there were no test substance concentrations with a mean number of revertants that was at least 2 times greater than the mean of concurrent vehicle control, or there was no concentration-related increase in the mean revertants per plate in that same strain.

Results not meeting criteria for positive or negative classification were evaluated using scientific judgment and experience and may have been reported as **EQUIVOCAL**.

RESULTS AND DISCUSSION

H-24119 was evaluated in the bacterial reverse mutation assay using *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, and *Escherichia coli* strain WP2 *uvrA* (pKM101) in the presence and absence of an exogenous metabolic activation system (Aroclor®-induced rat liver S9). The trial was performed using the plate incorporation method to evaluate the mutagenic potential of the test substance. Sterile water was chosen as the test substance solvent, diluent, and negative control.

In this study, concentrations of 5, 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate were evaluated in comparison to negative (solvent) controls. Mean positive control values measured as revertants per plate exhibited greater than a three-fold increase over the means of the respective negative control values for each tester strain (Tables 1-5). All tester strains exhibited appropriate phenotypic characteristics. The mean number of revertants observed in the negative control of each strain was within the prescribed acceptable range.

No precipitate was observed in any of the *Salmonella* strains or in the *Escherichia coli* strain. (Tables 1 - 5). No toxicity was observed as evidenced by a thinning of the microcolony background lawns or as a concentration-related reduction in the mean number of revertant colonies per plate. There were no test substance concentrations with a mean number of revertants that were 2 times greater than the mean of the concurrent vehicle control, nor was there a concentration-related increase in the mean revertants per plate in any strain (Tables 1-5).

CONCLUSIONS

Under the conditions of this study, no evidence of mutagenic activity was detected. The test substance was negative.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

REFERENCES

1. Maron, D. M. and B. N. Ames (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **113**, 173-215.

2. Green, M.H.L. and W. J. Muriel (1976). Mutagen testing using TRP^+ reversion in *Escherichia coli*. *Mutat. Res.* **38**, 3-32.

3. Gatehouse, D.G., Haworth, S., Cebula, T., Gocke, E., Kier, L., Matsushima, T., Melcion, C., Nohmi, T., Venitt, S. and E. Zeiger (1994). Recommendations for the Performance of Bacterial Mutation Assays. *Mutat. Res.* **312,** 217-233.

4. Levin, D. E., E. Yamasaki, and B. N. Ames (1982). A new *Salmonella* tester strain, TA97, for the detection of frameshift mutagens. A run of cytosines as a mutational hot spot. *Mutat. Res.* **94**, 315-330.

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6. Ames, B. N., F. D. Lee, and W. E. Durston (1973). An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc. Natl. Acad. Sci. USA* **70**, 782-786.

7. McCann, J., N. E. Springarn, J. Kobori, and B. N. Ames (1975). Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. *Proc. Natl. Acad. Sci. USA* **72,** 979-983.

ABBREVIATIONS FOR TABLES

Evidence for test substance toxicity to the bacteria was documented by recording the appearance of the plates and background lawn using the following key:

T0	**Normal**, background microcolony lawn appeared normal.
T1	**Slightly reduced**, background microcolony lawn was noticeably thinner.
T2	**Moderately reduced**, background lawn was markedly thinner resulting in an increase in the size of microcolonies compared to the vehicle control plate(s).
T3	**Severely reduced**, background lawn was distinguished by an extreme thinning resulting in an increase in the size of the microcolonies compared to the vehicle control plate(s). Microcolonies were seen readily by the unaided eye and were greatly enlarged relative to controls.
T4	**Absent**, plate(s) were distinguished by a complete lack of any microcolony lawn over a majority of the area of the plate(s).

Formation of a precipitate by the test material was documented using the following key:

P0	**No precipitate,** no precipitate observed.
P1	**Microscopic precipitate**, precipitate present that did not interfere with background lawn evaluation or automated colony counting.
P2	**Non-interfering precipitate,** precipitate present that was visible to the naked eye that did not interfere with automated colony counting.
P3	**Interfering precipitate,** precipitate present that required plate to be counted by hand.
P4	**Heavy interfering precipitate**, precipitate present that prevented accurate colony counting and obscured the background lawn requiring plate rejection (R).

Additional abbreviations may include the following:

N	Absence of any noteworthy observation
R	Plate rejected

Positive controls were abbreviated as follows:

[redacted]

TABLES

TABLE 1

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* TA97A

Concentration H-24119 (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Mean (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	125	133	132	130 (4)	T0,P0
5.0	141	155	134	143 (11)	T0,P0
10.0	142	127	135	135 (8)	T0,P0
50.0	131	142	147	140 (8)	T0,P0
100.0	134	123	159	139 (18)	T0,P0
500.0	120	139	144	134 (13)	T0,P0
1000.0	139	153	131	141 (11)	T0,P0
2500.0	162	141	130	144 (16)	T0,P0
5000.0	161	139	136	145 (14)	T0,P0
[redacted] 2 µg/plate	2067	1730	2177	1991 (233)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	156	152	158	155 (3)	T0,P0
5.0	136	138	131	135 (4)	T0,P0
10.0	149	137	120	135 (15)	T0,P0
50.0	127	158	148	144 (16)	T0,P0
100.0	165	141	153	153 (12)	T0,P0
500.0	131	150	169	150 (19)	T0,P0
1000.0	155	155	163	158 (5)	T0,P0
2500.0	144	141	158	148 (9)	T0,P0
5000.0	144	163	125	144 (19)	T0,P0
[redacted] 20 µg/plate	1985	1610	1721	1772 (193)	N

TABLE 2

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* TA98

Concentration H-24119 (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Mean (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	16	24	15	18 (5)	T0,P0
5.0	14	21	17	17 (4)	T0,P0
10.0	16	13	26	18 (7)	T0,P0
50.0	16	23	22	20 (4)	T0,P0
100.0	14	18	19	17 (3)	T0,P0
500.0	24	21	29	25 (4)	T0,P0
1000.0	22	21	23	22 (1)	T0,P0
2500.0	18	17	18	18 (1)	T0,P0
5000.0	17	27	20	21 (5)	T0,P0
[redacted]25 µg/plate	1048	1115	1054	1072 (37)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	34	34	28	32 (3)	T0,P0
5.0	33	25	32	30 (4)	T0,P0
10.0	40	24	31	32 (8)	T0,P0
50.0	24	15	34	24 (10)	T0,P0
100.0	27	22	28	26 (3)	T0,P0
500.0	29	20	26	25 (5)	T0,P0
1000.0	29	23	27	26 (3)	T0,P0
2500.0	33	18	18	23 (9)	T0,P0
5000.0	30	28	29	29 (1)	T0,P0
[redacted]2 µg/plate	993	1041	972	1002 (35)	N

TABLE 3

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* TA100

Concentration H-24119 (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Mean (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	181	183	176	180 (4)	T0,P0
5.0	166	185	207	186 (21)	T0,P0
10.0	179	183	204	189 (13)	T0,P0
50.0	173	166	218	186 (28)	T0,P0
100.0	178	213	198	196 (18)	T0,P0
500.0	195	170	181	182 (13)	T0,P0
1000.0	182	184	177	181 (4)	T0,P0
2500.0	188	206	181	192 (13)	T0,P0
5000.0	203	189	187	193 (9)	T0,P0
[redacted] µg/plate	1774	1927	1557	1753 (186)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	179	174	176	176 (3)	T0,P0
5.0	202	196	193	197 (5)	T0,P0
10.0	180	192	201	191 (11)	T0,P0
50.0	205	193	189	196 (8)	T0,P0
100.0	223	224	184	210 (23)	T0,P0
500.0	211	171	195	192 (20)	T0,P0
1000.0	204	205	203	204 (1)	T0,P0
2500.0	197	190	173	187 (12)	T0,P0
5000.0	170	184	180	178 (7)	T0,P0
[redacted] µg/plate	1294	1523	1432	1416 (115)	N

TABLE 4

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* TA1535

Concentration H-24119 (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Mean (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	7	9	10	9 (2)	T0,P0
5.0	7	12	10	10 (3)	T0,P0
10.0	7	13	4	8 (5)	T0,P0
50.0	11	5	13	10 (4)	T0,P0
100.0	9	9	4	7 (3)	T0,P0
500.0	5	10	11	9 (3)	T0,P0
1000.0	10	13	6	10 (4)	T0,P0
2500.0	9	12	7	9 (3)	T0,P0
5000.0	8	8	12	9 (2)	T0,P0
[redacted] 2 µg/plate	232	274	253	253 (21)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	13	11	13	12 (1)	T0,P0
5.0	17	14	12	14 (3)	T0,P0
10.0	16	10	9	12 (4)	T0,P0
50.0	12	16	12	13 (2)	T0,P0
100.0	16	12	9	12 (4)	T0,P0
500.0	12	8	16	12 (4)	T0,P0
1000.0	16	12	8	12 (4)	T0,P0
2500.0	15	12	11	13 (2)	T0,P0
5000.0	12	16	16	15 (2)	T0,P0
[redacted] 2 µg/plate	178	189	225	197 (25)	N

TABLE 5

MUTAGENIC ACTIVITY IN *ESCHERICHIA COLI* WP2 *UVRA* (PKM101)

Concentration H-24119 (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Mean (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	124	103	91	106 (17)	T0,P0
5.0	134	70	87	97 (33)	T0,P0
10.0	143	118	107	123 (18)	T0,P0
50.0	136	104	107	116 (18)	T0,P0
100.0	140	136	124	133 (8)	T0,P0
500.0	127	109	104	113 (12)	T0,P0
1000.0	139	88	108	112 (26)	T0,P0
2500.0	113	101	88	101 (13)	T0,P0
5000.0	151	84	103	113 (35)	T0,P0
[redacted] 2 µg/plate	1500	1227	1246	1324 (152)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	156	180	134	157 (23)	T0,P0
5.0	137	173	138	149 (21)	T0,P0
10.0	147	169	174	163 (14)	T0,P0
50.0	168	186	161	172 (13)	T0,P0
100.0	160	174	147	160 (14)	T0,P0
500.0	169	147	176	164 (15)	T0,P0
1000.0	185	142	156	161 (22)	T0,P0
2500.0	174	190	185	183 (8)	T0,P0
5000.0	178	175	169	174 (5)	T0,P0
[redacted] 25 µg/plate	1329	1632	1591	1517 (164)	N

TABLE 6

HISTORICAL CONTROL DATA[a]

Tester Strain Control [Positive Control[b]]	Exogenous Metabolic Activation System	Mean (S.D.)	Range Minimum-Maximum
S. typhimurium TA100			
Negative	Absent	122 (29)	54 – 218
Negative	Present	128 (29)	65 – 253
Positive [NAAZ-2]	Absent	872 (274)	339 – 2604
Positive [2AA-1]	Present	1187 (476)	94 – 2682
S. typhimurium TA 1535			
Negative	Absent	15 (6)	4 – 46
Negative	Present	14 (6)	4 – 39
Positive [NAAZ-2]	Absent	618 (199)	127 – 1270
Positive [2AA-2]	Present	362 (136)	44 – 1323
S. typhimurium TA97a			
Negative	Absent	103 (18)	59 – 164
Negative	Present	124 (26)	67 – 196
Positive [ICR 191-2]	Absent	1822 (662)	476 – 3359
Positive [DMBA-20]	Present	1534 (559)	563 – 2773
Positive [2AA-1]	Present	960 (348)	308 – 2281
S. typhimurium TA98			
Negative	Absent	22 (7)	7 – 47
Negative	Present	26 (7)	11 – 53
Positive [2NF-25]	Absent	1403 (372)	567 – 2774
Positive [2AA-2]	Present	1552 (598)	250 – 3114
E. coli WP2 *uvrA* (pKM101)			
Negative	Absent	148 (29)	82 – 221
Negative	Present	167 (29)	93 – 255
Positive [MMS-1000]	Absent	1656 (449)	208 – 2453
Positive [ENNG-2]	Absent	1627 (317)	1049 – 2253
Positive [2AA-25]	Present	1577 (450)	485 – 2484
Positive [2AA-250]	Present	1682 (372)	929 – 2230

a Historical data for tester strains used in the reported study. Data are based on studies reported during the period 1996 to 1998. Data include all control solvents or diluents, metabolic activation systems based on Aroclor®-induced rat liver S9, and all forms of study modification (e.g., plate incorporation, pre-incubation/gas, waste water).

[redacted]

Company Sanitized. Does not contain TSCA CBI