TRADE SECRET

Study Title

H-23005: Bacterial Reverse Mutation Test

LABORATORY PROJECT ID: HL-1998-01316

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STUDY COMPLETED ON: March 13, 1998

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with TSCA (40 CFR 792) Good Laboratory Practice Standards and OECD Principles of Good Laboratory Practice (C(81)30(Final), Annex 2), with the following exception:

1) Expired Oxoid nutrient plates were used in trial 1 for a portion of the phenotype confirmations. The plates were used a few days beyond their expiration, and all supported growth of viable bacteria, as was necessary for the phenotypic confirmations.

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

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

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

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

This deviation did not affect the validity or the integrity of the study.

Submitter/Sponsor: E. I. du Pont de Nemours and Company

Study Director: N. Larry Gladnick 3/13/98

N. Lawrence Gladnick, B.A.
Toxicology Associate

Date

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QUALITY ASSURANCE DOCUMENTATION

Haskell Sample Number(s):

23005

Dates of Inspections:

Conduct: January 21, 1998

Records, Reports: February 26-27, and March 2, 1998

Dates Findings Reported to:

Study Director: March 2, 1998

Management: March 13, 1998

Reported by: Paul Jeffrey Chapman 3/13/98

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Date

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CERTIFICATION

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

Reported Approved and Issued by Study Director: N. Larry Gladnick 3/13/98

N. Lawrence Gladnick, B.A.
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Date

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STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes:
- H-23005
- [redacted]
- [redacted]

Structure: 

Haskell Number: 23005

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Tan liquid

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
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Study Initiated/Completed: January 12, 1998 / (see report cover page)

In-Life Initiated/Completed: January 16, 1998 / February 6, 1998

STUDY PERSONNEL

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SUMMARY

The active ingredient of the test substance, H-23005, 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 test was performed in two phases. The first phase consisted of a preliminary toxicity assay that was used to establish test substance solubility in the selected solvent and the concentration range for the mutagenicity assay. The second phase, consisting of the initial and independent repeat assays, evaluated the mutagenic potential of the test substance.

Concentrations of 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate, adjusted for the active ingredient of the test substance, using the *Salmonella* strains and *Escherichia coli* strain, were evaluated in comparison to negative (solvent) controls. In a second independent repeat assay, these same concentrations, adjusted for the active ingredient of the test substance, were tested in comparison to negative (solvent) controls.

Under the conditions of this study, no evidence of mutagenic activity was detected in either of two independent trials. The test substance was negative.

INTRODUCTION

This study evaluated the mutagenic potential of the test substance, H-23005, 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 point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs.[1,2,3] 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 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.[4,5] 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

[redacted] The study was designed to comply with:

- U.S. EPA, Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guidelines (Subpart H, 40 CFR Part 799.9510 [1997]);
- Guidelines of the Organisation for Economic Cooperation and Development (OECD Guidelines for Testing of Chemicals, No. 471/472 "Bacterial Reverse Mutation Test" (Draft Updated Guideline, February, 1997));

The study design met or exceeded the above guidelines 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 or TA1537 (OECD).
- *E. coli* WP2 *uvrA* (pKM101) was the only *E. coli* tester strain used (OECD).

These exceptions did not affect study validity for the following reasons: 1) *Salmonella typhimurium* tester strain TA97a has improved growth properties and has been shown to be more sensitive to frameshift mutagens; 2) In the absence of clear evidence to suspect that the test substance was a DNA cross-linking agent, *E. coli* WP2 *uvrA* (pKM101) was used for an assessment of test substance mutagenicity at AT base pairs. 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 is H-23005. Additional information regarding the test substance is found on the study information page of this report. The test substance was assumed stable during this assay and no evidence of instability was observed.

2. Negative and Positive Controls

Based on information supplied by the sponsor and solubility testing, sterile water was chosen as the test substance solvent, diluent, and negative control. The negative control was assumed stable during this study, and no evidence of instability was observed. Any impurities were not expected to have interfered with the study. Positive controls included the following:

Positive Control Substances	Chemical Abstracts Service Registry Number
2-Nitrofluorene (2NF)	[CAS # 607-57-8]
2-Aminoanthracene (2AA)	[CAS # 613-13-8]
Sodium azide (NAAZ)	[CAS # 26628-22-8]
ICR 191 Acridine mutagen (ICR 191)	[CAS # 17070-45-0]
9,10-Dimethyl-1,2-benzanthracene (DMBA)	[CAS # 57-97-6]
N-Ethyl-*N*-nitro-*N*-nitroguanidine (ENNG)	[CAS # 4245-77-6]

Deionized water was the solvent for NAAZ and ICR 191. The solvent for 2AA, 2NF, DMBA, and ENNG was dimethyl sulfoxide (DMSO). The positive controls were assumed stable in this study and no evidence of instability was observed. Any impurities were not expected to have interfered with the study.

C. TESTER STRAIN CHARACTERIZATION, STORAGE, AND CULTURE

1. Tester Strain Characterization

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	Excision Repair	LPS	R-factor (pKM101) Plasmid	pAQ1 Plasmid
S. typhimurium TA97a	*his*D6610	Δ*uvrB*	*rfa*	Present	Absent
S. typhimurium TA98	*his*D3052	Δ*uvrB*	*rfa*	Present	Absent
S. typhimurium TA100	*his*G46	Δ*uvrB*	*rfa*	Present	Absent
S. typhimurium TA102	*his*G428	(+)	*rfa*	Present	Present
S. typhimurium TA1535	*his*G46	Δ*uvrB*	*rfa*	Absent	Absent
E. coli WP2 *uvrA* (pKM101)	*trpE*	Δ*uvrA*	NA	Present	Absent

LPS=lipopolysaccharide; NA=Not applicable; (+) proficient

The deletion (Δ) in *uvrB* (a gene that codes for one protein involved in DNA excision repair) increases bacterial sensitivity to several mutagens.[6] The *uvrB* trait is confirmed by demonstrating an increased bacterial sensitivity to ultraviolet light. Because this deletion also extends through a gene needed for biotin biosynthesis, the bacteria 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. 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, conferring tetracycline resistance, is tested at aliquot date for TA102.

Although some testing guidelines require *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 in place of TA97 due to its improved growth properties.[8] Although some testing guidelines require *E. coli* WP2 *uvrA*, in the absence of data indicating a difference in sensitivity between the plasmid and non-plasmid containing strains, *E. coli* WP2 *uvrA* (pKM101) was used to assess bacterial mutagenicity.[3] *E. coli* WP2 *uvrA* (pKM101) possess a deletion (Δ) in *uvrA* (involved in DNA excision repair), that is confirmed by demonstrating an increased sensitivity to ultraviolet light.

2. Tester Strain Storage and Culture

All bacterial strains were stored frozen in approximately 8% (v/v) DMSO in Oxoid Nutrient Broth No. 2 at approximately -70°C. Overnight cultures were prepared by inoculating 20 mL of Oxoid Nutrient Broth No. 2 with 0.1 mL of a thawed bacterial suspension and incubating at ~37°C with shaking. Overnight cultures were then stored on ice until used for mutagenesis assays. Bacterial strain phenotypes were confirmed concurrently with each trial. Results for reported trials demonstrated the appropriate responses.

D. METABOLIC ACTIVATION SYSTEM

Because bacterial strains lack many of the enzymes required to convert various 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 (1983). 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.) for five 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 systems, the prepared S9 mixture was streaked on nutrient agar capable of supporting the growth of viable bacteria. The amount of Aroclor®-induced rat liver S9 in the exogenous metabolic activation system was as follows:

4.0 mg Aroclor®-induced rat liver S9 protein ~10 % [v/v] / mL
exogenous metabolic activation system

The component concentrations in the exogenous metabolic activation system were as follows:

8 mM $MgCl_2$
33 mM KCl
5 mM Glucose-6-phosphate (as a sodium salt)
4 mM $NADP^+$ (as sodium salt)
100 mM Sodium phosphate buffer pH 7.4

E. CONCENTRATION SELECTION

In accordance with testing guidelines, the highest concentration evaluated in this study was approximately 167 mg/mL or 5000 µg/plate of the active ingredient of the test substance.

F. STABILITY AND CONCENTRATION VERIFICATION

Solutions of the test substance were prepared immediately prior to treatment and were presumed to be stable under the conditions of the study. Treatment and control solutions were not analyzed for concentration, uniformity, or stability. Top agar was not assayed for stability nor were the concentrations of the test substance or control articles since this assessment was not considered necessary to achieve the objectives of the study. Test substance sterility was confirmed by streaking the stock concentration in triplicate onto the surface of nutrient agar plates capable of supporting the growth of viable bacteria.

G. BACTERIAL MUTAGENICITY ASSAYS

This study consisted of two independent trials. For each trial, three replicates were plated for each tester strain in the presence and absence of the exogenous metabolic activation system (Aroclor®-induced rat liver S9) 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 [0.6-0.7% agar (w/v) and 0.5-0.6% NaCl (w/v)] 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 of a 0.4% [w/v] glucose with Davis salts, purchased from MOLTOX™). Treatments in the absence of the metabolic activation system were 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 approximately 37°C for about 48 hours. When necessary, plates were refrigerated at approximately 4°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 entirely by automated colony counter unless otherwise documented in the study records. Plates were also examined for test substance precipitation. Plates with test substance precipitation that interfered with the automated colony counting were counted manually when possible.

H. STATISTICAL ANALYSIS

Data for each tester strain were evaluated independently. For each tester strain, the average number of revertants and the standard deviation at each concentration with or without an exogenous metabolic activation system were calculated.

I. 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 for reported data was as follows:

1. Tester Strain Phenotypes and Spontaneous Reversion

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). All *S. typhimurium* tester strain cultures must have exhibited L-histidine dependent growth and *E. coli* WP2 *uvrA* (pKM101) 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.

Based on published data and historical data collected at the testing facility, all tester strain cultures must have exhibited a characteristic number of spontaneous revertants per plate in the absence of the test substance. The mean revertants per plate in the presence or absence of exogenous metabolic activation systems was 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) (101-227). Historical data collected for the testing facility is presented in Table 11.

2. Tester Strain Titer

To ensure that appropriate numbers of bacteria were plated, all tester strain culture titers must have been 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 (solvent) control value for each tester strain and condition.

4. Toxicity

A minimum of three non-toxic concentration levels was required to classify the test substance. A concentration level was considered toxic if it caused a >50 % reduction in the mean number of revertants per plate relative to the mean of the concurrent negative control value.

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 two data points, or if variability between replicate plates was judged excessive. Excessive variability was determined using scientific judgment.

An assay for an individual strain was rejected if the negative control was rejected, if mutagenic activity was absent on all positive control plates, or if the tester strain failed to exhibit the appropriate phenotypes.

Only those trials that met the criteria of acceptability were included in this report. All data will be maintained in the study records.

J. CLASSIFICATION GUIDELINES

A test substance was classified as **POSITIVE** (i.e., mutagenic) if the average number of revertants in any strain at any test substance concentration was at least two times greater than the average number of revertants in 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 an average number of revertants that were at least two times greater than the average number of revertants in the concurrent vehicle control; or there was no positive concentration-related increase in the mean revertants per plate in that same strain. In consultation with the sponsor, negative results may be confirmed.

Results not meeting criteria for positive or negative classification were evaluated using scientific judgment and experience. A study conclusion may be reported as **EQUIVOCAL**.

RESULTS AND DISCUSSION

The active ingredient of the test substance, H-23005, 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 test was performed in two phases. The first phase consisted of a preliminary toxicity assay that was used to establish test substance solubility in the selected solvent and the concentration range for the mutagenicity assay. The second phase, consisting of the initial and independent repeat assays, evaluated the mutagenic potential of the test substance, with the test concentrations adjusted so that the active ingredient was assessed at 5000 μg/plate.

Solubility and Preliminary Toxicity Assay

Based on information supplied by the sponsor on the test substance evaluation form and preliminary solubility determinations, sterile water was chosen as the test substance solvent, diluent, and negative control. The test substance dissolved completely at 50 mg/mL for the preliminary toxicity assays, and at approximately 167 mg/mL for the two independent trials. The solution appeared off-white and slightly opaque.

The preliminary toxicity assay was used to establish a concentration range over which the test substance was assayed. Concentrations of 10, 50, 100, 250, 500, 1000, 2500, and 5000 μg/plate of the test substance were evaluated, one plate per dose, in comparison to negative (solvent) controls. *Salmonella typhimurium* strains TA97a and TA100 and the *Escherichia coli* strain WP2 *uvrA* (pKM101) in the presence and absence of an exogenous metabolic activation system (Aroclor®-induced rat liver S9) were used in the preliminary toxicity assay.

Trial 1 and Trial 2

The results from the preliminary toxicity assay showed no significant decrease in the number of colonies at the highest concentration (5000 μg/plate) assessed in comparison to negative solvent controls in the *Salmonella* strains or *Escherichia coli* strain. Toxicity was not observed at any concentration, and only microscopic precipitate was observed.

The active ingredient of the test substance was to be evaluated at the highest concentration set forth by applicable test guidelines in comparison to negative controls. Data from both independent trials were consistent with that of the preliminary toxicity test. No toxicity was observed at any concentration. In the second independent repeat assay, the same concentrations were tested as in the first assay in comparison to negative controls for all bacteria. Precipitate was observed in all assays with all strains, in plated concentrations as low as 100 μg/plate and up to 5000 μg/plate. Data are presented in Tables 1-10.

CONCLUSION

Under the conditions of this study, no evidence of mutagenic activity was detected in either of two independent trials. 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. Ames, B.N., *et al.* (1975). Methods for Detecting Carcinogens and Mutagens with the *Salmonella*/Mammalian-Microsome Mutagenicity Test. *Mutation Research*, **31**, 347-364.

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8. Levin, D. E., *et al.* (1982). A new Salmonella tester strain, TA97a, for the detection of frameshift mutagens. A run of cytosines as a mutational hot spot. *Mutation Research* **94**, 315-330.

TABLES

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 substance 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 include the following:

N Absence of any noteworthy observation

TABLE 1

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA97a IN TRIAL 1

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	126	154	129	136 (15)	T0,P0
10.0	130	157	162	150 (17)	T0,P0
50.0	137	119	162	139 (22)	T0,P0
100.0	144	163	160	156 (10)	T0,P0
500.0	154	170	132	152 (19)	T0,P1
1000.0	151	140	122	138 (15)	T0,P1
2500.0	141	188	161	163 (24)	T0,P2
5000.0	118	139	135	131 (11)	T0,P2
ICR 191					
2 µg/plate	2421	2478	2450	2450 (29)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	154	161	143	153 (9)	T0,P0
10.0	177	174	147	166 (17)	T0,P0
50.0	176	153	145	158 (16)	T0,P0
100.0	166	163	159	163 (4)	T0,P0
500.0	172	156	174	167 (10)	T0,P1
1000.0	168	178	166	171 (6)	T0,P1
2500.0	170	165	156	164 (7)	T0,P2
5000.0	138	151	134	141 (9)	T0,P3
DMBA					
20 µg/plate	1720	1703	1742	1722 (20)	N

TABLE 2

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA97a IN TRIAL 2

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	111	134	149	131 (19)	T0,P0
10.0	177	130	121	143 (30)	T0,P0
50.0	137	145	140	141 (4)	T0,P0
100.0	139	155	146	147 (8)	T0,P1
500.0	151	152	141	148 (6)	T0,P1
1000.0	164	165	168	166 (2)	T0,P1
2500.0	124	109	124	119 (9)	T0,P2
5000.0	130	127	105	121 (14)	T0,P2
ICR 191					
2 µg/plate	799	852	786	812 (35)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	135	154	159	149 (13)	T0,P0
10.0	150	147	143	147 (4)	T0,P0
50.0	147	165	147	153 (10)	T0,P0
100.0	187	178	157	174 (15)	T0,P1
500.0	157	153	177	162 (13)	T0,P1
1000.0	162	138	163	154 (14)	T0,P1
2500.0	140	141	150	144 (6)	T0,P2
5000.0	136	125	150	137 (13)	T0,P3
DMBA					
20 µg/plate	853	928	849	877 (45)	N

TABLE 3

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA98 IN TRIAL 1

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	14	13	15	14 (1)	T0,P0
10.0	17	17	24	19 (4)	T0,P0
50.0	16	19	17	17 (2)	T0,P0
100.0	15	16	12	14 (2)	T0,P0
500.0	13	19	13	15 (3)	T0,P1
1000.0	13	21	18	17 (4)	T0,P1
2500.0	21	20	20	20 (1)	T0,P2
5000.0	8	10	11	10 (2)	T1,P2
2NF 25 µg/plate	1046	960	1054	1020 (52)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	29	24	35	29 (6)	T0,P0
10.0	32	31	28	30 (2)	T0,P0
50.0	29	35	28	31 (4)	T0,P0
100.0	29	32	24	28 (4)	T0,P0
500.0	25	21	24	23 (2)	T0,P1
1000.0	28	22	12	21 (8)	T0,P1
2500.0	20	22	12	18 (5)	T0,P2
5000.0	16	10	10	12 (3)	T0,P3
2AA 2 µg/plate	972	1012	1050	1011 (39)	N

TABLE 4

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA98 IN TRIAL 2

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	25	23	23	24 (1)	T0,P0
10.0	20	27	27	25 (4)	T0,P0
50.0	19	14	12	15 (4)	T0,P0
100.0	19	28	14	20 (7)	T0,P0
500.0	19	14	23	19 (5)	T0,P1
1000.0	20	16	22	19 (3)	T0,P1
2500.0	20	20	23	21 (2)	T0,P2
5000.0	20	21	23	21 (2)	T0,P2
2NF 25 µg/plate	1651	1574	1561	1595 (49)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	18	13	28	20 (8)	T0,P0
10.0	19	14	18	17 (3)	T0,P0
50.0	18	14	15	16 (2)	T0,P0
100.0	26	25	20	24 (3)	T0,P1
500.0	19	13	21	18 (4)	T0,P1
1000.0	27	17	22	22 (5)	T0,P2
2500.0	24	23	24	24 (1)	T0,P2
5000.0	15	17	21	18 (3)	T0,P3
2AA 2 µg/plate	748	710	794	751 (42)	N

TABLE 5

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA100 IN TRIAL 1

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	115	106	100	107 (8)	T0,P0
10.0	93	91	95	93 (2)	T0,P0
50.0	103	92	86	94 (9)	T0,P0
100.0	126	104	104	111 (13)	T0,P0
500.0	137	99	91	109 (25)	T0,P1
1000.0	90	96	106	97 (8)	T0,P1
2500.0	103	89	87	93 (9)	T0,P2
5000.0	95	68	76	80 (14)	T0,P2
NAAZ					
2 µg/plate	617	696	679	664 (42)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	122	98	129	116 (16)	T0,P0
10.0	124	107	100	110 (12)	T0,P0
50.0	121	115	106	114 (8)	T0,P0
100.0	107	100	111	106 (6)	T0,P1
500.0	102	128	115	115 (13)	T0,P1
1000.0	101	115	135	117 (17)	T0,P2
2500.0	110	120	109	113 (6)	T0,P2
5000.0	80	99	118	99 (19)	T0,P3
2AA					
1 µg/plate	362	358	430	383 (40)	N

TABLE 6

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA100 IN TRIAL 2

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	102	130	103	112 (16)	T0,P0
10.0	125	97	126	116 (16)	T0,P0
50.0	118	99	103	107 (10)	T0,P0
100.0	128	101	108	112 (14)	T0,P1
500.0	143	113	134	130 (15)	T0,P1
1000.0	135	126	131	131 (5)	T0,P1
2500.0	123	114	122	120 (5)	T0,P2
5000.0	101	113	97	104 (8)	T0,P2
NAAZ					
2 µg/plate	843	854	832	843 (11)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	117	136	139	131 (12)	T0,P0
10.0	124	111	149	128 (19)	T0,P0
50.0	125	145	127	132 (11)	T0,P0
100.0	158	135	116	136 (21)	T0,P1
500.0	133	117	139	130 (11)	T0,P1
1000.0	117	129	130	125 (7)	T0,P1
2500.0	148	106	117	124 (22)	T0,P2
5000.0	112	96	102	103 (8)	T0,P3
2AA					
2 µg/plate	896	914	885	898 (15)	N

TABLE 7

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA1535 IN TRIAL 1

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	12	14	14	13 (1)	T0,P0
10.0	20	14	6	13 (7)	T0,P0
50.0	9	11	15	12 (3)	T0,P0
100.0	10	12	14	12 (2)	T0,P0
500.0	10	13	9	11 (2)	T0,P1
1000.0	16	9	15	13 (4)	T0,P1
2500.0	11	11	10	11 (1)	T0,P2
5000.0	9	10	12	10 (2)	T0,P2
NAAZ					
2 µg/plate	479	561	585	542 (56)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	6	7	5	6 (1)	T0,P0
10.0	4	11	12	9 (4)	T0,P0
50.0	12	6	8	9 (3)	T0,P0
100.0	13	9	9	10 (2)	T0,P1
500.0	8	8	15	10 (4)	T0,P1
1000.0	10	7	7	8 (2)	T0,P2
2500.0	12	10	10	11 (1)	T0,P2
5000.0	9	8	8	8 (1)	T0,P3
2AA					
2 µg/plate	170	183	223	192 (28)	N

TABLE 8

MUTAGENIC ACTIVITY OF H-23005 IN *SALMONELLA TYPHIMURIUM* TA1535 IN TRIAL 2

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	9	6	10	8 (2)	T0,P0
10.0	9	13	6	9 (4)	T0,P0
50.0	10	8	9	9 (1)	T0,P0
100.0	9	15	9	11 (3)	T0,P0
500.0	13	12	8	11 (3)	T0,P1
1000.0	9	8	8	8 (1)	T0,P1
2500.0	12	12	10	11 (1)	T0,P2
5000.0	7	15	13	12 (4)	T0,P2
NAAZ					
2 µg/plate	628	571	623	607 (32)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	4	9	10	8 (3)	T0,P0
10.0	12	16	12	13 (2)	T0,P0
50.0	7	11	17	12 (5)	T0,P0
100.0	7	9	7	8 (1)	T0,P1
500.0	14	13	11	13 (2)	T0,P1
1000.0	11	9	12	11 (2)	T0,P1
2500.0	10	9	14	11 (3)	T0,P2
5000.0	8	8	12	9 (2)	T0,P3
2AA					
2 µg/plate	79	96	123	99 (22)	N

TABLE 9

MUTAGENIC ACTIVITY OF H-23005 IN *ESCHERICHIA COLI* WP2 *uvrA* (pKM101) IN TRIAL 1

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	137	168	136	147 (18)	T0,P0
10.0	134	126	132	131 (4)	T0,P0
50.0	131	151	173	152 (21)	T0,P0
100.0	162	142	96	133 (34)	T0,P0
500.0	137	158	120	138 (19)	T0,P1
1000.0	131	118	120	123 (7)	T0,P1
2500.0	140	114	167	140 (27)	T0,P2
5000.0	150	164	132	149 (16)	T0,P2
ENNG					
2 µg/plate	1237	1384	1302	1308 (74)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	182	154	185	174 (17)	T0,P0
10.0	154	171	214	180 (31)	T0,P0
50.0	151	140	146	146 (6)	T0,P0
100.0	142	170	156	156 (14)	T0,P1
500.0	126	134	146	135 (10)	T0,P1
1000.0	175	148	150	158 (15)	T0,P2
2500.0	156	165	153	158 (6)	T0,P2
5000.0	133	129	130	131 (2)	T0,P2
2AA					
25 µg/plate	2222	2276	2172	2223 (52)	N

TABLE 10

MUTAGENIC ACTIVITY OF H-23005 IN *ESCHERICHIA COLI* WP2 *uvrA* (pKM101) IN TRIAL 2

Concentration H-23005 (active ingredient) (µg/plate)	Revertants Plate 1	Plate 2	Plate 3	Average (S.D.)	Observations
A. WITHOUT METABOLIC ACTIVATION					
0.0	121	114	106	114 (8)	T0,P0
10.0	127	127	137	130 (6)	T0,P0
50.0	126	154	131	137 (15)	T0,P0
100.0	137	152	137	142 (9)	T0,P0
500.0	137	129	128	131 (5)	T0,P1
1000.0	142	123	137	134 (10)	T0,P1
2500.0	113	135	116	121 (12)	T0,P2
5000.0	99	102	101	101 (2)	T0,P2
ENNG					
2 µg/plate	1342	1296	1330	1323 (24)	N
B. WITH METABOLIC ACTIVATION (Aroclor®-induced rat liver S9)					
0.0	147	153	154	151 (4)	T0,P0
10.0	178	167	156	167 (11)	T0,P0
50.0	187	188	164	180 (14)	T0,P0
100.0	180	172	169	174 (6)	T0,P1
500.0	171	176	202	183 (17)	T0,P1
1000.0	220	182	163	188 (29)	T0,P2
2500.0	137	144	167	149 (16)	T0,P2
5000.0	153	160	174	162 (11)	T0,P3
2AA					
25 µg/plate	1030	1161	1214	1135 (95)	N

TABLE 11

HISTORICAL CONTROL DATA[1]

Tester Strain Control [Positive Control[2]]	Exogenous Metabolic Activation System	Mean (S.D.)	Range Minimum-Maximum
S. typhimurium TA100			
Negative	Absent	121 (31)	54 – 218
Negative	Present	128 (32)	65 – 253
Positive [NAAZ-2]	Absent	853 (252)	339 – 2604
Positive [2AA-1]	Present	1211 (457)	94 – 2682
S. typhimurium TA 1535			
Negative	Absent	16 (6)	7 – 46
Negative	Present	15 (6)	5 – 39
Positive [NAAZ-2]	Absent	576 (173)	206 – 1156
Positive [2AA-2]	Present	387 (134)	55 – 1323
S. typhimurium TA97a			
Negative	Absent	101 (18)	59 – 151
Negative	Present	117 (25)	67 – 196
Positive [ICR 191-2]	Absent	1747 (671)	476 – 3209
Positive [DMBA-20]	Present	1357 (334)	702 – 1683
Positive [2AA-1]	Present	941 (316)	308 – 2035
S. typhimurium TA98			
Negative	Absent	23 (7)	9 – 46
Negative	Present	26 (7)	11 – 49
Positive [2NF-25]	Absent	1391 (364)	567 – 2373
Positive [2AA-2]	Present	1656 (544)	437 – 3114
E. coli WP2 *uvrA* (pKM101)			
Negative	Absent	156 (27)	90 – 221
Negative	Present	172 (28)	93 – 255
Positive [MMS-1000]	Absent	1656 (446)	208 – 2453
Positive [ENNG-2]	Absent	1662 (155)	1353–1918
Positive [2AA-25]	Present	1592 (487)	485 – 2484
Positive [2AA-250]	Present	1682 (372)	929 – 2230

[1]Historical data for tester strains used in the reported study. Data are based on studies reported during the period 1996 up to and including November 26, 1997. 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).

[2]Abbreviations for positive controls: NAAZ (sodium azide); 2AA (2-aminoanthracene); 2NF (2-nitrofluorene); MMS (methyl methanesulfonate); ICR 191 (ICR 191 Acridine mutagen); DMBA (9,10-dimethyl-1,2-benzanthracene), ENNG (*N*-ethyl-*N*-nitro-*N*-nitrosoguanidine). The number following abbreviation is the microgram (μg) amount per plate or vial used for the positive control.