

Note: The test substance is identified on page 7 of Dupont-19713 RV1 as “Crude Industrial Grade HFPODA Ammonium Salt; H-27529”. In a letter to USEPA dated February 6, 2018, Chemours stated that H-27529 was the internal designation given by DuPont to the ammonium salt. Thus, Chemours believes that the test substance is indeed the ammonium salt.

TRADE SECRET

Study Title

H-27529:
Bacterial Reverse Mutation Test

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

OECD Guidelines for the Testing of Chemicals
Section 4 (Part 471) (1998)

EC Commission Directive 2000/32/EC Annex 4D-B.13/14
Number L 136

AUTHOR: Abby Myhre, B.S.

ORIGINAL REPORT

COMPLETED: May 31, 2006

REPORT REVISION 1

COMPLETED: February 22, 2008

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-19713

WORK REQUEST NUMBER: 16540

SERVICE CODE NUMBER: 500

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices, except for the items documented below. None of the items listed impact the validity of the study.

1. This study was conducted using test substance that was not characterized.
2. Neither the vehicle nor the positive controls were characterized by the testing facility or the sponsor. However, both the vehicle and positive controls were purchased from a reputable vendor and showed results consistent with historical control data.
3. The concentrations of the positive control and test substance dose solutions were not confirmed analytically; however, the solutions were prepared by trained personnel to ensure the accuracy of the concentrations.

Study Director: _____



Abby Myhre, B.S.
Associate Scientist

22 Feb 2008

Date

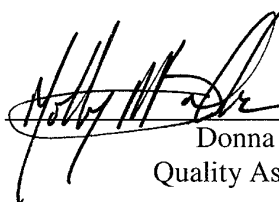
QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 16540
Study Code Number: 500

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

<i>Phase Audited</i>	<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
Protocol:	March 17, 2006	March 20, 2006	March 21, 2006
Conduct:	March 29, 2006	March 29, 2006	March 29, 2006
Report/Records:	May 26, 2006	May 26, 2006	May 30, 2006
Report Revision 1:	February 21, 2008	February 21, 2008	February 21, 2008

Reported by:

 For Donna M. Johnston
Donna M. Johnston
Quality Assurance Auditor

21 February 2008
Date

CERTIFICATION

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

Reviewed and Approved by: E. Maria - Donner 21 - Feb - 2008
E. Maria Donner, Ph.D.
Senior Research Toxicologist and Manager
Date

Issued by Study Director: Abby Myhre 22-Feb-2008
Abby Myhre, B.S.
Associate Scientist
Date

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STUDY INFORMATION

Substance Tested: • Crude Industrial Grade HFODA Ammonium Salt
• H-27529

Haskell Number: 27529

Composition: 85.4-85.8 wt%
Balance is water

Purity: See composition, above

Physical Characteristics: Clear liquid

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

Study Initiated/Completed: March 16, 2006 / (see report cover page)

Experimental Start/Termination: March 22, 2006 / April 3, 2006

REASON FOR REVISION 1

The name of the substance tested was revised on the Study Information Page.

SUMMARY

The test substance, H-27529, was evaluated for mutagenicity in the Bacterial Reverse Mutation Test using the plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2uvrA were tested in the presence and absence of an exogenous metabolic activation system (Aroclor-induced rat liver S9).

The test was performed in 2 phases. The first phase was the toxicity-mutation test which established the dose range for the mutagenicity test, and provided a preliminary mutagenicity evaluation. The second phase was the mutagenicity test which evaluated and confirmed the mutagenic potential of the test substance.

Sterile water was chosen as the dosing vehicle based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in water at 50 mg/mL, the highest concentration that was tested in the study.

In the toxicity-mutation test, the maximum dose evaluated was 5000 µg/plate. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels used in this test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 µg/plate. No positive mutagenic responses were observed at any dose level in any tester strain in either the presence or absence of S9 metabolic activation. No toxicity or test substance precipitation was observed at any dose level with any tester strain in either the presence or absence of S9 metabolic activation.

Based on the toxicity-mutation test, the maximum dose evaluated in the mutagenicity test was 5000 µg/plate for tester strains TA98, TA100, TA1535, TA1537, and WP2uvrA in the presence and absence of S9. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels used in this test were 333, 667, 1000, 3333, and 5000 µg/plate for all tester strains. The plate incorporation method was employed. No positive mutagenic responses were observed at any dose level or with any tester strain in either the presence or absence of S9 metabolic activation. No toxicity or test substance precipitation was observed at any dose level with any tester strain in either the presence or the absence of S9 metabolic activation.

All criteria for a valid study were met. Under the conditions of this study, H-27529 showed no evidence of mutagenicity in the Bacterial Reverse Mutation Test either in the presence or absence of Aroclor-induced rat liver S9. The test substance was concluded to be negative in this study.

INTRODUCTION

The objective of this study was to evaluate the test substance, H-27529, for its ability to induce reverse mutations at the histidine locus in the genome of *Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537), and at the tryptophan locus of the *Escherichia coli* strain WP2 *uvrA*. The assay was conducted with and without an exogenous S9 metabolic activation system.

MATERIALS AND METHODS

A. Testing Guidelines

This study was conducted in compliance with the following guidelines:

- U.S. EPA, OPPTS 870.5100: Bacterial Reverse Mutation Test, *Health Effects Test Guidelines* (1998)
- OECD, Section 4 (Part 471): Bacterial Reverse Mutation Test, *Guidelines for Testing of Chemicals* (1998)
- European Commission Directive 2000/32/EC of May 19, 2000, Annex 4D-B13/14. Mutagenicity - Reverse Mutation Test Bacteria. Number L 136

B. Test Substance and Controls

1. Identification

The test substance, H-27529, was a clear liquid. The test substance batch used for this study was assigned Haskell Identification Number 27529. Additional information regarding the test substance is found on the study information page of this report.

2. Sample Preparation, Stability, and Analytical Verification of Test Substance Concentrations

The sponsor-reported purity for H-27529 was 85.4-85.8% active ingredient. A correction factor of 85.4% was used for preparation of the dosing solutions. An analytical verification of the test substance concentrations was not conducted.

3. Controls

Negative:	sterile water (CAS# 7732-18-5, HPLC grade, Burdick & Jackson)
Positive (Moltox Inc.):	benzo[a]pyrene [CAS# 50-32-8] 4-nitroquinoline N-oxide [CAS# 56-57-5] acridine mutagen ICR-191 [CAS# 17070-45-0] sodium azide [CAS# 26628-22-8] 2-aminoanthracene [CAS# 613-13-8] 2-nitrofluorene [CAS# 607-57-8]

The positive controls were dissolved in dimethyl sulfoxide (DMSO, CAS# 67-68-6, 99.9% purity, EMD), except for sodium azide and ICR-191, which were dissolved in sterile water. The positive controls were assumed to be stable during this test and no evidence of instability was observed.

C. Test System

The tester strains were the *Salmonella typhimurium* histidine auxotroph tester strains TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* tryptophan auxotroph WP2uvrA.^(1,2,3) All tester strains were obtained from Moltox Inc. (Boone, North Carolina). The specific genotypes and phenotypic characterization of these strains were as follows:

Tester Strain	HIS/Trp Mutation	Additional Mutations		
		Repair	LPS	Plasmid
<i>S. typhimurium</i> TA98	<i>hisD3052</i>	$\Delta uvrB$	<i>rfa</i>	pKM101
<i>S. typhimurium</i> TA100	<i>hisG46</i>	$\Delta uvrB$	<i>rfa</i>	pKM101
<i>S. typhimurium</i> TA1535	<i>hisG46</i>	$\Delta uvrB$	<i>rfa</i>	--
<i>S. typhimurium</i> TA1537	<i>hisC3076</i>	$\Delta uvrB$	<i>rfa</i>	--
<i>Escherichia coli</i> WP2uvrA	<i>trpE</i>	$\Delta uvrA$	-	--

In addition to a mutation in either the histidine or tryptophan operons, the tester strains contain additional mutations that enhance their sensitivity to some mutagens. A mutation of either the *uvrA* or *uvrB* gene results in a deficient DNA excision repair system. Since the *uvrB* deletion extends through the *bio* gene, the *Salmonella typhimurium* tester strains also require the vitamin biotin for growth.

The *Salmonella typhimurium* tester strains also contain the *rfa* wall mutation which results in the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide (LPS) barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals such as those containing large ring systems that would otherwise be excluded by a normal intact cell wall.

Tester strains TA98 and TA100 also contain the pKM101 plasmid, which further increases the sensitivity of these strains to some mutagens.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independent (prototrophy) by frameshift mutagens. Tester strain TA100 is reverted by both frame shift and base substitution mutagens. Tester strains TA1535 and WP2*uvrA* are reverted from auxotrophy to prototrophy by base substitution mutagens.

D. Preparation and Storage of Tester Strain

Frozen permanent stocks of all tester strains were prepared by growing fresh overnight cultures with the addition of 0.09 mL DMSO per milliliter of culture. Aliquots were frozen in dry ice and stored at $\leq -70^{\circ}\text{C}$.

Master plates were prepared by streaking each tester strain from a frozen permanent stock onto either nutrient agar plates or minimal glucose agar plates. The minimal glucose agar plates were supplemented with either histidine and biotin or tryptophan, and for strains containing the pKM101 plasmid, ampicillin. Tester strain master plates were stored at $5 \pm 3^{\circ}\text{C}$.

Overnight cultures for use in the study were inoculated from the appropriate master plates. Cultures were placed in a shaker/incubator for overnight at 150 ± 50 rpm and $37 \pm 2^{\circ}\text{C}$. To ensure that appropriate numbers of bacteria are plated, the length of incubation was determined by spectrophotometric monitoring of culture density.

E. Confirmation of Tester Strain Genotype

Tester strain cultures were checked for the following genetic markers on the day of the preparation of master plates.

The histidine requirement was tested by comparing the growth of each *Salmonella* tester strain on a histidine/biotin-supplemented minimum glucose agar plate with their growth on a biotin-only minimum glucose agar plate.

The tryptophan requirement was tested by comparing the growth of WP2*uvrA* strain on a tryptophan-supplemented minimum glucose agar plate with their growth on a minimum glucose agar plate.

For the *Salmonella* tester strains the presence of the *rfa* wall mutation was confirmed by demonstration of the sensitivity of the cultures to crystal violet.

The presence of *uvrA* and *uvrB* mutation was demonstrated by their sensitivity to ultraviolet light of the tester strains.

The presence of the pKM101 plasmid was confirmed for cultures of tester strains TA98 and TA100 by demonstration of resistance to ampicillin.

F. Experimental Design and Methodology

1. Solubility Determination and Selection of Vehicle

Based on the solubility of the test substance and compatibility with the target cells, sterile water was chosen as the test substance solvent.

2. Exogenous Metabolic Activation and Sham Mix

Liver homogenate (S9, average protein concentration: 38.9 mg/mL) prepared from male Sprague-Dawley rats induced with Aroclor 1254 was purchased commercially (Moltox Inc., Boone, North Carolina).

The S9 was thawed and the 10% S9 mix prepared immediately prior to its use. The S9 mix was held on ice at all times. The S9 mix contained proportionate volumes of the following components:

HPLC-grade water	2.4 mL
0.825 M KCl/0.2 M MgCl ₂	0.4 mL
0.2 M phosphate buffer, pH 7.4	5.0 mL
0.25 M glucose-6-phosphate	0.2 mL
0.04 M NADP	1.0 mL
S9	1.0 mL
Total Volume	10 mL

The sham mix was 100 mM phosphate buffer at pH 7.4.

3. Controls

a. Negative Controls

Sterile water, as the negative control, was plated for each tester strain with and without S9 activation.

b. Positive Controls

Tester Strain	S9 Mix	Positive Control	µg per plate
TA98	+	benzo[a]pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	Acridine mutagen ICR-191	2.0
WP2 _{uvrA}	+	2-aminoanthracene	25.0
WP2 _{uvrA}	-	4-nitroquinoline-N-oxide	1.0

c. Sterility Controls

100 µL of the most concentrated test substance dilution (50 mg/mL) was plated to check the sterility of the test substance. The S9 and sham mix were checked for sterility by plating 0.5 mL on selective agar plates.

4. Plate Identification, Frequency, and Route of Administration

Each plate was labeled with the work request number, service code, Haskell number, treatment date, and plate number. The plate number signifies a positive control, a negative control or a sample plate, and tester strain, the presence or absence of S9 metabolic activation, dose level, and replicate.

In the non-activated assays, 0.5 mL of sham mix and 100 µL of vehicle, test substance dilution, or positive control were added to pre-heated (45–48°C) glass culture tubes containing 2 mL of selective top agar, followed by 100 µL of tester strain.

In the S9-activated assays, 100 µL of the vehicle, test substance dilution, or positive control were added to pre-heated (45–48°C) glass culture tubes containing 2 mL of selective top agar, followed by 100 µL of tester strain and 0.5 mL of S9 mix.

All mixtures were vortexed and overlaid onto the surface of minimum glucose agar plate. After the overlay solidified, the plates were inverted and incubated for approximately 48 hours at $37 \pm 2^\circ\text{C}$. Plates were stored at approximately 4°C before being counted. All toxicity-mutation test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in duplicate. All mutagenicity test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in triplicate.

5. Dose Level Determination

The dose levels for the toxicity-mutation test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 µg per plate. The dose levels for the mutagenicity test were 333, 667, 1000, 3333, and 5000 µg per plate.

6. Toxicity-Mutation Test, Mutagenicity Test, and Test Method

The test substance was evaluated along with negative and positive controls using tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA* with and without S9 activation. The plate incorporation method was employed. Dose levels for the mutagenicity test were chosen from the toxicity-mutation test results and were listed in the study records and the final report. The toxicity-mutation test used duplicate plates for each dose level and the mutagenicity test used triplicate plates.

7. Scoring

Revertant colonies were counted with either an automated colony counter (Sorcerer, Perceptive Instruments Ltd., Suffolk, United Kingdom), or manually. The appearance of the bacterial background lawn was assessed for test substance toxicity and precipitation. Precipitation was assessed by visual examination.

G. Criteria for Determination of a Valid Test

1. Tester Strain Integrity

To demonstrate the presence of the *rfa* mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to crystal violet. To demonstrate the presence of the *uvrA* and *uvrB* mutations, all tester strains cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the pKM101 plasmid, tester strain cultures of TA98 and TA100 must exhibit resistance to ampicillin.

2. Tester Strain Culture Density

To ensure that appropriate numbers of bacteria are plated, all tester strain culture densities must be equal to or greater than 0.3×10^9 cells per milliliter.

3. Negative Control Values

The tester strain cultures must exhibit a characteristic mean number of spontaneous revertants per plate when plated along with the negative (vehicle) control under selective conditions. The acceptable ranges for the mean values of negative controls are as follows:

Tester Strain	Negative Control Range
TA98	8-60
TA100	60-240
TA1535	4-45
TA1537	2-25
WP2 _{uvrA}	5-60

4. Positive Control Values

Each mean positive control value must exhibit at least a 3.0-fold increase over the respective mean negative (vehicle) control value for each tester strain.

5. Toxicity

A minimum of three non-toxic scorable dose levels are required to validate the study. A dose level is considered toxic if it causes:

- A >50% reduction in the mean number of revertants per plate relative to the mean negative control value and exhibits a dose-dependent drop in the revertant count, **or**
- A reduction in the background lawn.

In the event that less than 3 non-toxic dose levels are achieved, the affected portion of the test will be repeated with an appropriate change in dose levels.

6. Data Point Rejection

- A single data point may have been rejected if contamination or excessive toxicity was seen on a treatment plate. A single data point may also have been rejected if excessive precipitate on the plate prevented accurate colony counting.
- A negative control data point may have been rejected if it fell outside the acceptable spontaneous mutation range.

H. Evaluation of Test Results

Criteria for a positive response:

1. Strains TA1535 and TA1537

Data will be judged positive if the increase in mean revertants at the highest numerical dose response is ≥ 3.0 -fold the mean concurrent negative control value (vehicle control). This increase in the mean number of revertants per plate must be accompanied by a dose response associated with increasing concentrations of the test substance.

2. Strains TA98, TA100 and WP2*uvrA*

Data sets will be judged positive if the increase in mean revertants at the highest numerical dose response is ≥ 2.0 -fold the mean concurrent negative control value (vehicle control). This increase in the mean number of revertants per plate must be accompanied by a dose response to increasing concentrations of the test substance.

I. Data Presentation

For each tester strain, the mean of the number of revertants and the standard deviations were calculated.

RESULTS AND DISCUSSION

A. Solubility

The test substance formed a clear and soluble solution in water at 50 mg/mL, the highest concentration that was tested in the study.

B. Sterility Controls

No contaminant colonies were observed on the sterility plates for the most concentrated test substance dilution (50 mg/mL) and the S9 and sham mixes.

C. Toxicity-Mutation Test

(Tables 1-10 and 21-22)

In the toxicity-mutation test, the maximum dose evaluated was 5000 $\mu\text{g}/\text{plate}$. This dose was achieved using a concentration of 50 mg/mL and a 100 μL plating aliquot. The dose levels used in this test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 $\mu\text{g}/\text{plate}$. No positive mutagenic responses were observed at any dose level in any tester strain in either the presence or absence of S9 metabolic activation. No toxicity or test substance precipitation was observed at any dose level with any tester strain in either the presence or absence of S9 metabolic activation.

D. Mutagenicity Test

(Tables 11-20 and 23-24)

Based on the toxicity-mutation test, the maximum dose evaluated in the mutagenicity test was 5000 $\mu\text{g}/\text{plate}$ for tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA* in the presence and absence of S9. This dose was achieved using a concentration of 50 mg/mL and a 100 μL plating aliquot. The dose levels used in this test were 333, 667, 1000, 3333, and 5000 $\mu\text{g}/\text{plate}$ for all tester strains. The plate incorporation method was employed. No positive mutagenic responses were observed at any dose level or with any tester strain in either the presence or absence of S9 metabolic activation. No toxicity or test substance precipitation was observed at

any dose level with any tester strain in either the presence or the absence of S9 metabolic activation.

CONCLUSIONS

All criteria for a valid study were met. Under the conditions of this study, H-27529 showed no evidence of mutagenicity in the Bacterial Reverse Mutation Test either in the presence or absence of Aroclor-induced rat liver S9. The test substance was concluded to be negative in this study.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), 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., McCann, J., and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research* 31, 347-364.
2. Maron, D.M., and Ames, B. (1983). Revised Methods for the Salmonella Mutagenicity Test. *Mutation Research* 113, 173-215.
3. Wilcox, P., Naidoo, A., Wedd, D.J., and Gatehouse, D.G. (1990). Comparison of Salmonella typhimurium TA102 with Escherichia coli WP2 tester strains. *Mutagenesis* 5, 285-291.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

C.V.	coefficient of variation
SD	standard deviation

Bacterial Background Lawn Evaluation Code – Evidence for test substance toxicity to the bacteria will be documented by recording the appearance of the background lawn using the following code:

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

Test Substance Precipitation Code – Formation of a precipitate by the test substance will be documented using the following code:

P0	No precipitate , no precipitate observed.
P1	Microscopic precipitate , precipitate present which does not interfere with background lawn evaluation or automated colony counting.
P2	Non-interfering precipitate , precipitate present that is visible to the naked eye that does not interfere with automated colony counting.
P3	Interfering precipitate , precipitate present that requires plate to be counted by hand.
P4	Heavy interfering precipitate , precipitate present that prevents accurate colony counting and obscures the background lawn requiring plate rejection (R).

Lost Plate Justification Code:

L0	The loss of this test substance-treated plate does not invalidate the results since the remaining plate at this dose level and the remaining treated plates are also comparable to the negative control.
L1	The loss of this vehicle control plate does not invalidate the results since the remaining vehicle control plate is consistent with the historical negative control value for this condition.
L2	The loss of this positive control plate does not invalidate the results since the remaining positive control plate is consistent with the historical positive control value for this condition.
L3	The loss of this test substance-treated plate does not invalidate the results since the remaining plate is consistent with the remaining treated plates.
L4	The loss of this test substance-treated plate does not invalidate the results since the remaining plate at this dose level is comparable to the negative control.
L5	The loss of this untreated control plate does not invalidate the results since the remaining plate at the dose level is comparable to the vehicle control and is consistent with the historical negative control value for this condition.

Table 1
Toxicity-mutation test in *Salmonella typhimurium* TA98 without S9

Strain:	TA98	Experiment No:		T-1	
Rat Liver S9:	Absent	Cell Titer (cells/mL):		4.77×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		22-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	121	16	T0,P0	15	2
	122	13	T0,P0		
Positive Control ^b	123	177	T0,P0	177	1
	124	176	T0,P0		
33.3	125	22	T0,P0	18	6
	126	13	T0,P0		
66.7	127	16	T0,P0	16	0
	128	16	T0,P0		
100	129	18	T0,P0	19	1
	130	20	T0,P0		
333	131	20	T0,P0	17	5
	132	13	T0,P0		
667	133	27	T0,P0	26	2
	134	24	T0,P0		
1000	135	10	T0,P0	14	6
	136	18	T0,P0		
3333	137	11	T0,P0	15	6
	138	19	T0,P0		
5000	139	25	T0,P0	22	4
	140	19	T0,P0		

^a Sterile Water

^b 1.0 µg/plate 2-nitrofluorene

Table 2
Toxicity-mutation test in *Salmonella typhimurium* TA100 without S9

Strain:	TA100	Experiment No:		T-1	
Rat Liver S9:	Absent	Cell Titer (cells/mL):		4.10×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		22-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	141	144	T0,P0	130	20
	142	116	T0,P0		
Positive Control ^b	143	1143	T0,P0	1167	33
	144	1190	T0,P0		
33.3	145	113	T0,P0	119	8
	146	125	T0,P0		
66.7	147	119	T0,P0	118	2
	148	116	T0,P0		
100	149	128	T0,P0	129	1
	150	129	T0,P0		
333	151	113	T0,P0	104	13
	152	95	T0,P0		
667	153	105	T0,P0	101	6
	154	96	T0,P0		
1000	155	116	T0,P0	110	8
	156	104	T0,P0		
3333	157	108	T0,P0	115	9
	158	121	T0,P0		
5000	159	110	T0,P0	117	10
	160	124	T0,P0		

^a Sterile Water

^b 2.0 µg/plate sodium azide

Table 3
Toxicity-mutation test in *Salmonella typhimurium* TA1535 without S9

Strain:	TA1535		Experiment No:	T-1	
Rat Liver S9:	Absent		Cell Titer (cells/mL):	4.77×10 ⁸	
Plating Aliquot:	100 µL		Date Plated:	22-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	161	8	T0,P0	10	2
	162	11	T0,P0		
Positive Control ^b	163	866	T0,P0	878	17
	164	890	T0,P0		
33.3	165	11	T0,P0	9	4
	166	6	T0,P0		
66.7	167	5	T0,P0	7	3
	168	9	T0,P0		
100	169	15	T0,P0	10	7
	170	5	T0,P0		
333	171	10	T0,P0	12	2
	172	13	T0,P0		
667	173	13	T0,P0	11	3
	174	9	T0,P0		
1000	175	11	T0,P0	15	5
	176	18	T0,P0		
3333	177	14	T0,P0	12	4
	178	9	T0,P0		
5000	179	8	T0,P0	8	0
	180	8	T0,P0		

^a Sterile Water

^b 2.0 µg/plate sodium azide

Table 4
Toxicity-mutation test in *Salmonella typhimurium* TA1537 without S9

Strain:	TA1537		Experiment No:	T-1	
Rat Liver S9:	Absent		Cell Titer (cells/mL):	5.92×10 ⁸	
Plating Aliquot:	100 μL		Date Plated:	22-Mar-06	
Dose (μg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	181	11	T0,P0	8	4
	182	5	T0,P0		
Positive Control ^b	183	1641	T0,P0	1568	103
	184	1495	T0,P0		
33.3	185	10	T0,P0	10	0
	186	10	T0,P0		
66.7	187	6	T0,P0	7	1
	188	8	T0,P0		
100	189	6	T0,P0	5	1
	190	4	T0,P0		
333	191	6	T0,P0	8	3
	192	10	T0,P0		
667	193	4	T0,P0	4	0
	194	4	T0,P0		
1000	195	6	T0,P0	10	5
	196	13	T0,P0		
3333	197	4	T0,P0	8	5
	198	11	T0,P0		
5000	199	8	T0,P0	7	1
	200	6	T0,P0		

^a Sterile Water

^b 2.0 µg/plate ICR-191

Table 5
Toxicity-mutation test in *Escherichia coli* WP2*uvrA* without S9

Strain:	WP2 _{uvrA}		Experiment No:	T-1	
Rat Liver S9:	Absent		Cell Titer (cells/mL):	4.03×10 ⁸	
Plating Aliquot:	100 μL		Date Plated:	22-Mar-06	
Dose (μg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	101	16	T0,P0	22	8
	102	28	T0,P0		
Positive Control ^b	103	518	T0,P0	528	13
	104	537	T0,P0		
33.3	105	37	T0,P0	33	6
	106	28	T0,P0		
66.7	107	30	T0,P0	39	13
	108	48	T0,P0		
100	109	22	T0,P0	25	4
	110	28	T0,P0		
333	111	38	T0,P0	31	11
	112	23	T0,P0		
667	113	27	T0,P0	33	8
	114	39	T0,P0		
1000	115	33	T0,P0	34	1
	116	35	T0,P0		
3333	117	33	T0,P0	38	6
	118	42	T0,P0		
5000	119	24	T0,P0	37	18
	120	49	T0,P0		

^a Sterile Water

^b 1.0 µg/plate 4-nitroquinoline-N-oxide

Table 6
Toxicity-mutation test in *Salmonella typhimurium* TA98 with S9

Strain:	TA98	Experiment No:	T-1		
Rat Liver S9:	Present	Cell Titer (cells/mL):	4.77×10 ⁸		
Plating Aliquot:	100 µL	Date Plated:	22-Mar-06		
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	21	39	T0,P0	35	6
	22	30	T0,P0		
Positive Control ^b	23	295	T0,P0	335	57
	24	375	T0,P0		
33.3	25	23	T0,P0	26	4
	26	29	T0,P0		
66.7	27	27	T0,P0	27	0
	28	27	T0,P0		
100	29	22	T0,P0	20	3
	30	18	T0,P0		
333	31	29	T0,P0	27	3
	32	25	T0,P0		
667	33	23	T0,P0	31	11
	34	39	T0,P0		
1000	35	25	T0,P0	22	5
	36	18	T0,P0		
3333	37	16	T0,P0	21	6
	38	25	T0,P0		
5000	39	24	T0,P0	25	1
	40	25	T0,P0		

^a Sterile Water

^b 2.5 µg/plate benzo(a)pyrene

Table 7
Toxicity-mutation test in *Salmonella typhimurium* TA100 with S9

Strain:	TA100	Experiment No:	T-1		
Rat Liver S9:	Present	Cell Titer (cells/mL):	4.10×10 ⁸		
Plating Aliquot:	100 µL	Date Plated:	22-Mar-06		
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	41	109	T0,P0	102	10
	42	95	T0,P0		
Positive Control ^b	43	1894	T0,P0	1932	54
	44	1970	T0,P0		
33.3	45	110	T0,P0	107	4
	46	104	T0,P0		
66.7	47	101	T0,P0	105	6
	48	109	T0,P0		
100	49	106	T0,P0	118	17
	50	130	T0,P0		
333	51	128	T0,P0	124	6
	52	119	T0,P0		
667	53	99	T0,P0	110	15
	54	120	T0,P0		
1000	55	125	T0,P0	115	14
	56	105	T0,P0		
3333	57	137	T0,P0	138	1
	58	139	T0,P0		
5000	59	116	T0,P0	117	1
	60	118	T0,P0		

^a Sterile Water

^b 2.5 µg/plate 2-aminoanthracene

Table 8
Toxicity-mutation test in *Salmonella typhimurium* TA1535 with S9

Strain:	TA1535		Experiment No:	T-1	
Rat Liver S9:	Present		Cell Titer (cells/mL):	4.77×10 ⁸	
Plating Aliquot:	100 μL		Date Plated:	22-Mar-06	
Dose (μg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	61	10	T0,P0	12	2
	62	13	T0,P0		
Positive Control ^b	63	219	T0,P0	210	13
	64	200	T0,P0		
33.3	65	5	T0,P0	9	6
	66	13	T0,P0		
66.7	67	11	T0,P0	11	1
	68	10	T0,P0		
100	69	8	T0,P0	12	5
	70	15	T0,P0		
333	71	19	T0,P0	12	10
	72	5	T0,P0		
667	73	11	T0,P0	11	1
	74	10	T0,P0		
1000	75	11	T0,P0	12	1
	76	13	T0,P0		
3333	77	10	T0,P0	9	1
	78	8	T0,P0		
5000	79	10	T0,P0	15	6
	80	19	T0,P0		

^a Sterile Water

^b 2.5 µg/plate 2-aminoanthracene

Table 9
Toxicity-mutation test in *Salmonella typhimurium* TA1537 with S9

Strain:	TA1537	Experiment No:		T-1	
Rat Liver S9:	Present	Cell Titer (cells/mL):		5.92×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		22-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	81	11	T0,P0	11	0
	82	11	T0,P0		
Positive Control ^b	83	116	T0,P0	130	19
	84	143	T0,P0		
33.3	85	11	T0,P0	11	1
	86	10	T0,P0		
66.7	87	5	T0,P0	7	2
	88	8	T0,P0		
100	89	6	T0,P0	8	2
	90	9	T0,P0		
333	91	6	T0,P0	8	2
	92	9	T0,P0		
667	93	9	T0,P0	11	3
	94	13	T0,P0		
1000	95	6	T0,P0	9	4
	96	11	T0,P0		
3333	97	5	T0,P0	7	3
	98	9	T0,P0		
5000	99	10	T0,P0	12	2
	100	13	T0,P0		

^a Sterile Water

^b 2.5 µg/plate 2-aminoanthracene

Table 10
Toxicity-mutation test in *Escherichia coli* WP2uvrA with S9

Strain:	WP2uvrA		Experiment No:	T-1	
Rat Liver S9:	Present		Cell Titer (cells/mL):	4.03×10 ⁸	
Plating Aliquot:	100 μL		Date Plated:	22-Mar-06	
Dose (μg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	1	38	T0,P0	33	7
	2	28	T0,P0		
Positive Control ^b	3	373	T0,P0	373	1
	4	372	T0,P0		
33.3	5	57	T0,P0	46	16
	6	35	T0,P0		
66.7	7	46	T0,P0	43	4
	8	40	T0,P0		
100	9	42	T0,P0	37	7
	10	32	T0,P0		
333	11	23	T0,P0	28	6
	12	32	T0,P0		
667	13	47	T0,P0	43	6
	14	38	T0,P0		
1000	15	34	T0,P0	37	4
	16	40	T0,P0		
3333	17	29	T0,P0	37	11
	18	44	T0,P0		
5000	19	35	T0,P0	39	5
	20	42	T0,P0		

^a Sterile Water

^b 25 µg/plate 2-aminoanthracene

Table 11
Mutagenicity test in *Salmonella typhimurium* TA98 without S9

Strain:	TA98	Experiment No:		E-1	
Rat Liver S9:	Absent	Cell Titer (cells/mL):		5.04×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	127	11	T0,P0	14	4
	128	14	T0,P0		
	129	18	T0,P0		
Positive Control ^b	130	267	T0,P0	216	44
	131	191	T0,P0		
	132	190	T0,P0		
333	133	18	T0,P0	17	4
	134	13	T0,P0		
	135	20	T0,P0		
667	136	16	T0,P0	19	4
	137	19	T0,P0		
	138	23	T0,P0		
1000	139	16	T0,P0	17	3
	140	14	T0,P0		
	141	20	T0,P0		
3333	142	19	T0,P0	19	4
	143	16	T0,P0		
	144	23	T0,P0		
5000	145	19	T0,P0	17	3
	146	14	T0,P0		
	147	19	T0,P0		

^a Sterile Water

^b 1.0 µg/plate 2-nitrofluorene

Table 12
Mutagenicity test in *Salmonella typhimurium* TA100 without S9

Strain:	TA100	Experiment No:		E-1	
Rat Liver S9:	Absent	Cell Titer (cells/mL):		4.10×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	148	143	T0,P0	126	16
	149	125	T0,P0		
	150	111	T0,P0		
Positive Control ^b	151	995	T0,P0	1100	324
	152	842	T0,P0		
	153	1464	T0,P0		
333	154	121	T0,P0	118	8
	155	109	T0,P0		
	156	124	T0,P0		
667	157	97	T0,P0	107	9
	158	114	T0,P0		
	159	110	T0,P0		
1000	160	111	T0,P0	115	4
	161	118	T0,P0		
	162	115	T0,P0		
3333	163	108	T0,P0	112	8
	164	121	T0,P0		
	165	108	T0,P0		
5000	166	121	T0,P0	122	6
	167	116	T0,P0		
	168	128	T0,P0		

^a Sterile Water

^b 2.0 µg/plate sodium azide

Table 13
Mutagenicity test in *Salmonella typhimurium* TA1535 without S9

Strain:	TA1535	Experiment No:		E-1	
Rat Liver S9:	Absent	Cell Titer (cells/mL):		4.08×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	169	11	T0,P0	13	5
	170	9	T0,P0		
	171	18	T0,P0		
Positive Control ^b	172	750	T0,P0	758	35
	173	728	T0,P0		
	174	796	T0,P0		
333	175	11	T0,P0	11	3
	176	13	T0,P0		
	177	8	T0,P0		
667	178	11	T0,P0	11	3
	179	8	T0,P0		
	180	13	T0,P0		
1000	181	16	T0,P0	10	6
	182	11	T0,P0		
	183	4	T0,P0		
3333	184	11	T0,P0	12	1
	185	13	T0,P0		
	186	13	T0,P0		
5000	187	16	T0,P0	14	3
	188	15	T0,P0		
	189	10	T0,P0		

^a Sterile Water

^b 2.0 µg/plate sodium azide

Table 14
Mutagenicity test in *Salmonella typhimurium* TA1537 without S9

Strain:	TA1537	Experiment No:		E-1	
Rat Liver S9:	Absent	Cell Titer (cells/mL):		4.49×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	190	8	T0,P0	7	2
	191	5	T0,P0		
	192	8	T0,P0		
Positive Control ^b	193	1925	T0,P0	1755	291
	194	1922	T0,P0		
	195	1419	T0,P0		
333	196	13	T0,P0	8	5
	197	3	T0,P0		
	198	8	T0,P0		
667	199	6	T0,P0	7	3
	200	11	T0,P0		
	201	5	T0,P0		
1000	202	8	T0,P0	4	4
	203	0	T0,P0		
	204	4	T0,P0		
3333	205	5	T0,P0	8	4
	206	6	T0,P0		
	207	13	T0,P0		
5000	208	9	T0,P0	9	1
	209	10	T0,P0		
	210	8	T0,P0		

^a Sterile Water

^b 2.0 µg/plate ICR-191

Table 15
Mutagenicity test in *Escherichia coli* WP2uvrA without S9

Strain:	WP2 _{uvrA}		Experiment No:	E-1	
Rat Liver S9:	Absent		Cell Titer (cells/mL):	4.17×10 ⁸	
Plating Aliquot:	100 µL		Date Plated:	29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	106	28	T0,P0	31	6
	107	27	T0,P0		
	108	38	T0,P0		
Positive Control ^b	109	621	T0,P0	577	68
	110	611	T0,P0		
	111	499	T0,P0		
333	112	48	T0,P0	43	4
	113	40	T0,P0		
	114	42	T0,P0		
667	115	29	T0,P0	40	10
	116	48	T0,P0		
	117	43	T0,P0		
1000	118	33	T0,P0	27	10
	119	32	T0,P0		
	120	15	T0,P0		
3333	121	51	T0,P0	35	14
	122	24	T0,P0		
	123	30	T0,P0		
5000	124	27	T0,P0	32	5
	125	37	T0,P0		
	126	32	T0,P0		

^a Sterile Water

^b 1.0 µg/plate 4-nitroquinoline-N-oxide

Table 16
Mutagenicity test in *Salmonella typhimurium* TA98 with S9

Strain:	TA98	Experiment No:	E-1		
Rat Liver S9:	Present	Cell Titer (cells/mL):	5.04×10 ⁸		
Plating Aliquot:	100 µL	Date Plated:	29-Mar-06		
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	22	37	T0,P0	30	10
	23	19	T0,P0		
	24	35	T0,P0		
Positive Control ^b	25	329	T0,P0	361	29
	26	387	T0,P0		
	27	366	T0,P0		
333	28	19	T0,P0	21	3
	29	20	T0,P0		
	30	24	T0,P0		
667	31	34	T0,P0	30	6
	32	24	T0,P0		
	33	33	T0,P0		
1000	34	24	T0,P0	31	8
	35	40	T0,P0		
	36	29	T0,P0		
3333	37	40	T0,P0	36	3
	38	34	T0,P0		
	39	34	T0,P0		
5000	40	27	T0,P0	29	8
	41	22	T0,P0		
	42	37	T0,P0		

^a Sterile Water

^b 2.5 µg/plate benzo(a)pyrene

Table 17
Mutagenicity test in *Salmonella typhimurium* TA100 with S9

Strain:	TA100	Experiment No:		E-1	
Rat Liver S9:	Present	Cell Titer (cells/mL):		4.10×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	43	118	T0,P0	122	6
	44	120	T0,P0		
	45	129	T0,P0		
Positive Control ^b	46	1416	T0,P0	1504	159
	47	1409	T0,P0		
	48	1688	T0,P0		
333	49	130	T0,P0	126	11
	50	114	T0,P0		
	51	135	T0,P0		
667	52	110	T0,P0	119	10
	53	129	T0,P0		
	54	118	T0,P0		
1000	55	124	T0,P0	142	17
	56	144	T0,P0		
	57	158	T0,P0		
3333	58	116	T0,P0	126	15
	59	144	T0,P0		
	60	119	T0,P0		
5000	61	108	T0,P0	125	15
	62	132	T0,P0		
	63	135	T0,P0		

^a Sterile Water

^b 2.5 µg/plate 2-aminoanthracene

Table 18
Mutagenicity test in *Salmonella typhimurium* TA1535 with S9

Strain:	TA1535	Experiment No:	E-1		
Rat Liver S9:	Present	Cell Titer (cells/mL):	4.08×10 ⁸		
Plating Aliquot:	100 μL	Date Plated:	29-Mar-06		
Dose (μg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	64	14	T0,P0	12	4
	65	15	T0,P0		
	66	8	T0,P0		
Positive Control ^b	67	154	T0,P0	183	28
	68	209	T0,P0		
	69	186	T0,P0		
333	70	8	T0,P0	9	1
	71	10	T0,P0		
	72	8	T0,P0		
667	73	9	T0,P0	9	2
	74	11	T0,P0		
	75	8	T0,P0		
1000	76	18	T0,P0	13	7
	77	5	T0,P0		
	78	15	T0,P0		
3333	79	13	T0,P0	14	4
	80	11	T0,P0		
	81	18	T0,P0		
5000	82	14	T0,P0	12	2
	83	11	T0,P0		
	84	10	T0,P0		

^a Sterile Water

^b 2.5 µg/plate 2-aminoanthracene

Table 19
Mutagenicity test in *Salmonella typhimurium* TA1537 with S9

Strain:	TA1537	Experiment No:		E-1	
Rat Liver S9:	Present	Cell Titer (cells/mL):		4.49×10 ⁸	
Plating Aliquot:	100 µL	Date Plated:		29-Mar-06	
Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	85	9	T0,P0	6	2
	86	5	T0,P0		
	87	5	T0,P0		
Positive Control ^b	88	132	T0,P0	94	37
	89	58	T0,P0		
	90	92	T0,P0		
333	91	9	T0,P0	8	3
	92	5	T0,P0		
	93	10	T0,P0		
667	94	10	T0,P0	9	4
	95	5	T0,P0		
	96	13	T0,P0		
1000	97	5	T0,P0	5	1
	98	6	T0,P0		
	99	5	T0,P0		
3333	100	9	T0,P0	8	1
	101	8	T0,P0		
	102	8	T0,P0		
5000	103	10	T0,P0	8	3
	104	4	T0,P0		
	105	10	T0,P0		

^a Sterile Water

^b 2.5 µg/plate 2-aminoanthracene

Table 20
Mutagenicity test in *Escherichia coli* WP2uvrA with S9

Strain:	WP2uvrA		Experiment No:	E-1	
Rat Liver S9:	Present		Cell Titer (cells/mL):	4.17×10 ⁸	
Plating Aliquot:	100 μL		Date Plated:	29-Mar-06	
Dose (μg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle ^a	1	32	T0,P0	32	2
	2	34	T0,P0		
	3	30	T0,P0		
Positive Control ^b	4	390	T0,P0	418	29
	5	448	T0,P0		
	6	416	T0,P0		
333	7	38	T0,P0	41	2
	8	42	T0,P0		
	9	42	T0,P0		
667	10	44	T0,P0	37	6
	11	35	T0,P0		
	12	32	T0,P0		
1000	13	51	T0,P0	43	9
	14	44	T0,P0		
	15	34	T0,P0		
3333	16	33	T0,P0	34	1
	17	35	T0,P0		
	18	35	T0,P0		
5000	19	23	T0,P0	32	8
	20	39	T0,P0		
	21	34	T0,P0		

^a Sterile Water

^b 25 µg/plate 2-aminoanthracene

Table 21
Summary of the toxicity-mutation test without rat liver S9

Dose (µg/plate)	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2 _{uvrA}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	15	2	130	20	10	2	8	4	22	8
positive control	177	1	1167	33	878	17	1568	103	528	13
33.3	18	6	119	8	9	4	10	0	33	6
66.7	16	0	118	2	7	3	7	1	39	13
100	19	1	129	1	10	7	5	1	25	4
333	17	5	104	13	12	2	8	3	31	11
667	26	2	101	6	11	3	4	0	33	8
1000	14	6	110	8	15	5	10	5	34	1
3333	15	6	115	9	12	4	8	5	38	6
5000	22	4	117	10	8	0	7	1	37	18

Experiment No: T-1

Plate Aliquot: 100 µL

Table 22
Summary of the toxicity-mutation test with rat liver S9

Dose (µg/plate)	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2 _{uvrA}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	35	6	102	10	12	2	11	0	33	7
positive control	335	57	1932	54	210	13	130	19	373	1
33.3	26	4	107	4	9	6	11	1	46	16
66.7	27	0	105	6	11	1	7	2	43	4
100	20	3	118	17	12	5	8	2	37	7
333	27	3	124	6	12	10	8	2	28	6
667	31	11	110	15	11	1	11	3	43	6
1000	22	5	115	14	12	1	9	4	37	4
3333	21	6	138	1	9	1	7	3	37	11
5000	25	1	117	1	15	6	12	2	39	5

Experiment No: T-1

Plate Aliquot: 100 µL

Table 23
Summary of the mutagenicity test without rat liver S9

Dose (µg/plate)	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2 _{uvrA}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	14	4	126	16	13	5	7	2	31	6
positive control	216	44	1100	324	758	35	1755	291	577	68
333	17	4	118	8	11	3	8	5	43	4
667	19	4	107	9	11	3	7	3	40	10
1000	17	3	115	4	10	6	4	4	27	10
3333	19	4	112	8	12	1	8	4	35	14
5000	17	3	122	6	14	3	9	1	32	5

Experiment No: E-1

Plate Aliquot: 100 µL

Table 24
Summary of the mutagenicity test with rat liver S9

Dose (µg/plate)	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2 _{uvrA}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	30	10	122	6	12	4	6	2	32	2
positive control	361	29	1504	159	183	28	94	37	418	29
333	21	3	126	11	9	1	8	3	41	2
667	30	6	119	10	9	2	9	4	37	6
1000	31	8	142	17	13	7	5	1	43	9
3333	36	3	126	15	14	4	8	1	34	1
5000	29	8	125	15	12	2	8	3	32	8

Experiment No: E-1

Plate Aliquot: 100 µL

APPENDICES

Appendix A
Historical Control Data

HISTORICAL CONTROL DATA^a

Tester Strain	Exogenous Metabolic	Mean	(SD) ^c	Range	
Control [Positive Control ^b]	Activation System			Minimum	- Maximum
TA98					
Negative	Absent	22	(8)	6	- 47
Negative	Present	29	(9)	10	- 54
Positive [2NF-1]	Absent	190	(81)	49	- 361
Positive [2NF-25]	Absent	1403	(372)	567	- 2774
Positive [BAP-2.5]	Present	359	(65)	250	- 490
Positive [2AA-2]	Present	1552	(598)	250	- 3114
TA100					
Negative	Absent	126	(40)	54	- 253
Negative	Present	131	(32)	65	- 253
Positive [SA-2]	Absent	960	(219)	339	- 2604
Positive [2AA-1]	Present	1187	(476)	94	- 2682
Positive [2AA-2.5]	Present	2097	(418)	1525	- 3018
TA1535					
Negative	Absent	16	(7)	4	- 46
Negative	Present	14	(5)	4	- 39
Positive [SA-2]	Absent	752	(192)	127	- 1270
Positive [2AA-2.5]	Present	191	(72)	96	- 384
TA1537					
Negative	Absent	8	(3)	3	- 16
Negative	Present	11	(7)	3	- 29
Positive [ICR 191-2]	Absent	1649	(399)	1188	- 2573
Positive [2AA-2.5]	Present	100	(30)	48	- 170
WP2 <i>uvrA</i>					
Negative	Absent	44	(11)	21	- 64
Negative	Present	39	(16)	14	- 68
Positive [4NQO-1]	Absent	476	(95)	303	- 656
Positive [2AA-25]	Present	514	(107)	278	- 669

- a Historical data for tester strains used in the reported study. Data are based on studies reported since 1996. 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).
- b Abbreviations for positive controls: SA (sodium azide); 2AA (2-aminoanthracene); 2NF (2-nitrofluorene); ICR 191 (ICR 191 Acridine mutagen); 4NQO (4-nitroquinoline-N-oxide); BAP (benzo[a]pyrene). The number following abbreviation is the microgram (µg) amount per plate or vial used for the positive control.
- c SD = standard deviation