

REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

Bacterial Reverse Mutation Study

TEST ARTICLE NAME

Poly-ond (R) Plating

TEST ARTICLE IDENTIFICATION

Test Panels

NAMSA

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Summary

The test article, Poly-ond (R) Plating, was evaluated for the potential to cause mutagenic changes at the histidine locus of the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 or at the tryptophan locus of the *Escherichia coli* tester strain WP2uvrA. The study was conducted in the presence and absence of S9 metabolic activation based on ISO 10993-3, Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity and OECD 471, Guideline for Testing of Chemicals, Bacterial Reverse Mutation Test. The test article was extracted in dimethyl sulfoxide (DMSO) and saline (SC).

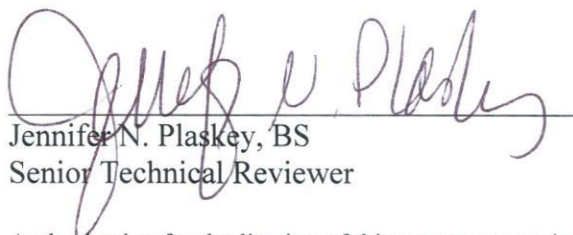
Tubes containing molten top agar were inoculated with culture from one of the five tester strains, along with the DMSO or saline test article extract. An aliquot of sterile water for injection or rat liver S9 homogenate, providing metabolic activation, was added. The mixture was poured across triplicate plates. Parallel testing was conducted with negative controls (extraction vehicle alone) and positive controls. The mean number of revertants for the test extract plates was compared to the mean number of revertants of the negative control plates for each of the five tester strains.


The DMSO and saline test article extracts were considered to be nonmutagenic to *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *E. coli* tester strain WP2uvrA.

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Table 3: Positive Control Article

Name:	Sodium azide, methyl methanesulfonate, 2-aminoanthracene, benzo[a]pyrene, 2-nitrofluorene, and ICR-191 were included as positive controls to verify assay performance.
Strength, Purity, Composition or Other Characteristics:	<p>Sodium azide: Purity: $\geq 99.5\%$; Composition: CAS No. 26628-22-8</p> <p>Methyl methanesulfonate: Purity: $\geq 99.9\%$; Composition: CAS No. 66-27-3</p> <p>2-aminoanthracene: Purity: $\geq 96\%$; Composition: CAS No. 613-13-8</p> <p>Benzo[a]pyrene: Purity: $\geq 96\%$; Composition: CAS No. 50-32-8</p> <p>2-Nitrofluorene: Purity: $\geq 97.5\%$; Composition: CAS No. 607-57-8</p> <p>ICR-191: Purity: Not less than 90%; Identity: Proton NMR spectrum is consistent with structure of CAS No. 17070-45-0;</p> <p>Composition: CAS No. 17070-45-0</p>

3. Test System

3.1 Test System

The bacterial reverse mutation assay detects point mutations, frameshifts and/or base pair substitutions. The strains of *Salmonella typhimurium* and *Escherichia coli* used in this assay are histidine and tryptophan auxotrophs, respectively, as defined by the conditionally lethal mutations in the appropriate operons. When these histidine (*his*⁻) or tryptophan (*trp*⁻) dependent cells are grown on a selection media (minimal E media with histidine or tryptophan in the media respectively), only those cells which revert to the histidine (*his*⁺) or tryptophan (*trp*⁺) phenotype are able to form colonies. The plated bacteria undergo a minimal number of cell divisions, which is essential for phenotype expression of mutations. The histidine and tryptophan allows all the plated bacteria to undergo replication and mutagenesis expression to occur. The *his*⁺ or *trp*⁺ revertants are readily discernable as colonies against the limited background growth of the *his*⁻ or *trp*⁻ cells. By utilizing several different tester strains, both base pair substitution mutations and frameshift mutations can be detected. The bacterial reverse mutation assay has been shown to be a sensitive, rapid and accurate indicator of the mutagenic activity of many materials including a wide range of chemical classes.

The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant. If a mutagen is added to the test system, the mutation rate is significantly increased.

Table 4: Test System Description

Tester Strain	<i>his/trp</i> Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	hisD3052, frameshift	<i>uvrB</i>	<i>rfa</i>	pKM101
TA100	hisG46, missense	<i>uvrB</i>	<i>rfa</i>	pKM101
TA1535	hisG46, missense	<i>uvrB</i>	<i>rfa</i>	-
TA1537	hisC3076, frameshift	<i>uvrB</i>	<i>rfa</i>	-
WP2 <i>uvrA</i>	trpE65, missense	<i>uvrA</i>	-	-

rfa = causes partial loss of the lipopolysaccharide (LPS) wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)

uvrB or *uvrA* = deficient DNA excision - repair system (i.e., ultraviolet sensitivity)

frameshift = base-pair addition/deletion

missense = base-pair substitution

pKM101 = plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

3.2 Metabolic Activation

Aroclor 1254 - induced rat liver (S9 homogenate) was purchased from Moltox (Boone, North Carolina) and used for metabolic activation. Just prior to use, the S9 homogenate was mixed with a buffer containing 0.4 M MgCl₂/1.65 M KCl, 1.0 M glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer, and sterile water for injection.

3.3 Preparation of Tester Strains

Cultures of *S. typhimurium* tester strain TA98, TA100, TA1535 and TA1537, and *E. coli* tester strain WP2*uvrA*, were inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 115-125 rpm for approximately 10-12 hours. Strain characteristics were verified and cell density was determined.

3.4 Negative Control

DMSO and saline (vehicles without test article) were tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested in the presence and absence of S9 activation. These data provided a base rate to which the number of revertant colonies that developed in each test plate was compared to determine whether the test article had significant mutagenic properties.

3.5 Positive Control

Known mutagens, benzo[a]pyrene and 2-nitrofluorene, were used as positive controls to demonstrate that *S. typhimurium* tester strain TA98 was sensitive to mutation to the wild type state. For *S. typhimurium* tester strains TA100 and TA1535, sodium azide and 2-aminoanthracene were used as positive controls. For *S. typhimurium* tester strain TA1537, 2-aminoanthracene and ICR-191 were used as positive controls. For *E. coli* tester strain WP2uvrA, 2-aminoanthracene and methyl methanesulfonate were used as positive controls.

Table 5: Positive Control Summary

Positive Control	Concentration	S9	Tester Strain
Benzo[a]pyrene	2.5 µg/plate	Presence	TA98
2-Nitrofluorene	5.0 µg/plate	Absence	
2-Aminoanthracene	2.5 µg/plate	Presence	TA100
Sodium Azide	20 µg/plate	Absence	
2-Aminoanthracene	2.5 µg/plate	Presence	TA1535
Sodium Azide	20 µg/plate	Absence	
2-Aminoanthracene	2.5 µg/plate	Presence	TA1537
ICR-191	2.0 µg/plate	Absence	
2-Aminoanthracene	20 µg/plate	Presence	WP2uvrA
Methyl methanesulfonate	3.25 mg/plate	Absence	

4. Method

4.1 Test Article and Negative Control Article Preparation

Only the plates were included in the preparation. The test article and the control (extraction vehicle without the test article) were subjected to the extraction conditions as described below. The extracts incubated at 70°C were continuously agitated during extraction.

Table 6: Extraction

Vehicle	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
DMSO	120 cm ² :20 mL	67.6 cm ²	11 mL	70°C for 24 hours
Saline	120 cm ² :20 mL	67.6 cm ²	11 mL	121°C for 1 hour

The following table contains a description of the test and control article extract conditions.

Table 7: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
DMSO	Before Extraction	Test	Colorless	Clear	No
		Negative Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Negative Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Negative Control	Colorless	Clear	No
Saline	Before Extraction	Test	Colorless	Clear	No
		Negative Control	Colorless	Clear	No
	After Extraction	Test	Yellow	Clear	No
		Negative Control	Colorless	Clear	No
	Prior to Use	Test	Yellow	Clear	Few, flakes, and orange
		Negative Control	Colorless	Clear	No

The test article remained visually unchanged following the extraction process. The extracts were maintained at room temperature for less than 6 hours before use. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

4.2 Test Procedure

4.2.1 Confirmation of Tester Strain Genotype

Tester strain cultures were checked for the following genetic markers prior to the evaluation of test and control plates:

4.2.2 *rfa* Mutation

For the *S. typhimurium* tester strains, the presence of the *rfa* mutation was confirmed by demonstration of the sensitivity of the culture to crystal violet. An aliquot of an overnight culture of each strain was streaked onto plates containing selective media, and a disk containing crystal violet was added. Sensitivity was demonstrated by inhibition of bacterial growth in a zone immediately surrounding the disk.

4.2.3 pKM101 Plasmid

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

4.2.4 Amino Acid Dependence

Amino acid dependence was confirmed for each tester strain. For the *S. typhimurium* tester strains, dependence on histidine was confirmed by demonstration that the strains were not viable in the absence of histidine. For *E. coli* tester strain WP2uvrA, dependence on tryptophan was confirmed by demonstration that the strain was not viable in the absence of tryptophan.

4.2.5 *uvrB/uvrA* Deletion

For the *S. typhimurium* tester strains, the presence of the *uvrB* deletion was confirmed by a demonstration of growth inhibition after exposure to ultraviolet radiation. For *E. coli* tester strain WP2*uvrA*, the presence of the *uvrA* deletion was confirmed by a demonstration of growth inhibition after exposure to ultraviolet radiation.

4.2.6 Standard Plate Incorporation Assay

Molten top agar was supplemented with histidine-biotin solution or tryptophan solution for the *S. typhimurium* or *E. coli* tester strains, respectively. This addition allowed the bacteria on the plate to undergo several divisions to produce a faint background lawn, visible to the naked eye, which could be examined under a darkfield colony counter. Separate tubes containing 2.0 mL of supplemented molten top agar were inoculated with 0.1 mL of culture for each of the five tester strains, and 0.1 mL of the DMSO or saline test article extract. A 0.5 mL aliquot of sterile water for injection or S9 homogenate, providing metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing was also conducted with each negative control and six positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) were prepared in triplicate as follows:

1. DMSO and saline test article extracts in the presence and absence of S9 activation
2. Negative controls in the presence and absence of S9 activation
3. Benzo[a]pyrene in the presence of S9 and 2-nitrofluorene in the absence of S9 activation with strain TA98
4. 2-Aminoanthracene in the presence of S9 and sodium azide in the absence of S9 activation with strain TA100
5. 2-Aminoanthracene in the presence of S9 and sodium azide in the absence of S9 activation with strain TA1535
6. 2-Aminoanthracene in the presence of S9 and ICR-191 in the absence of S9 activation with strain TA1537
7. 2-Aminoanthracene in the presence of S9 and methyl methanesulfonate in the absence of S9 activation with strain WP2*uvrA*

The plates were incubated at 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of revertants and standard deviation were determined. The mean number of revertants on the test plates was compared to the mean number of revertants on the negative control plates for each of the tester strains employed. The background lawn was recorded as follows:

Table 8: Background Lawn Description

Code	Description	Characteristics
1	Normal (N)	Distinguished by a healthy microcolony lawn.
2	Slightly reduced (SR)	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies.
3	Moderately reduced (MR)	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies.
4	Extremely reduced (ER)	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent (A)	Distinguished by a complete lack of any microcolony lawn over more than or equal to 90% of the plate.
6	Obscured by particulates (OP)	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
7	Non-interfering precipitate (NP)	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
8	Interfering precipitate (IP)	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total more than 10% of the revertant colony count (e.g., more than 3 particles on a plate with 30 revertants).

4.2.7 Sterility Verification

Sterility verification testing was performed as follows:

1. Each positive control, test extract, and negative control was transferred onto nutrient agar plates.
2. S9 Homogenate mix was transferred to a nutrient agar plate.
3. Sterile water for injection was transferred to a nutrient agar plate.
4. Each type of top agar was transferred to a nutrient agar plate.
5. One untreated Minimal E plate was evaluated.

Plates were incubated at 37°C for 2 days, after which all plates were evaluated for any signs of contamination.

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

5. Evaluation

For the DMSO and saline test extracts to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for strains TA98, TA100 and WP2uvrA and/or a 3-fold or greater increase in the number of mean revertants over the means obtained from the negative control for strains TA1535 and TA1537. Calculation of fold increase is the mean number of revertants of the test divided by the mean number of revertants for the respective negative control. Each positive control mean must have exhibited at least a 3-fold increase over the negative control mean, irrespective of vehicle, for all five tester strains. The negative control results of each tester strain should exhibit a characteristic number of spontaneous revertants based on historical data collected at NAMSA (Table 9). The historical ranges for each tester strain are updated annually.

Table 9: Spontaneous Reversion Rates

Species	Tester Strain	Number of Spontaneous Revertants	
		Without S9	With S9
<i>S. typhimurium</i>	TA98	13 - 50	15 - 50
	TA100	81 - 215	84 - 225
	TA1535	6 - 30	6 - 30
	TA1537	3 - 24	3 - 24
<i>E. coli</i>	WP2uvrA	12 - 70	14 - 75

The historical data for the controls are summarized in Table 10.

Table 10: Historical Data from January 5, 2016 to December 22, 2016

	Species	Tester Strain	Historical Ranges for Negative Control		Historical Ranges for Positive Control	
			Mean Revertant Rates ± SD	Number of Data Points	Mean Revertant Rates ± SD	Number of Data Points
Without S9	<i>S. typhimurium</i>	TA98	23 ± 6.2	261	1,626 ± 598.6	50
		TA100	147 ± 25.0	261	2,301 ± 557.7	50
		TA1535	13 ± 3.0	259	2,356 ± 691.0	50
		TA1537	7 ± 2.1	259	831 ± 338.8	50
	<i>E. coli</i>	WP2uvrA	27 ± 6.1	260	1,045 ± 293.0	50
With S9	<i>S. typhimurium</i>	TA98	26 ± 6.1	257	773 ± 211.7	50
		TA100	171 ± 29.2	257	2,288 ± 394.4	50
		TA1535	14 ± 3.2	258	517 ± 336.8	50
		TA1537	8 ± 2.2	258	335 ± 136.1	50
	<i>E. coli</i>	WP2uvrA	29 ± 6.7	257	671 ± 176.5	50

6. Results

6.1 Strain Characteristics

Salmonella typhimurium tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2uvrA exhibited appropriate genetic characteristics pertaining to this assay.

6.2 Sterility Verification

There was no contamination observed.

6.3 Tester Strain Revertants

The results are presented in Appendices 1 through 3. The background lawn appeared slightly reduced and normal for the DMSO and saline test extracts. In no case was there a 2-fold or greater increase in the mean number of revertants for tester strains TA98, TA100 and WP2uvrA or a 3-fold or greater increase in the mean number of revertants for tester strains TA1535 and TA1537 in the presence of DMSO and saline test article extracts (Table 11). The negative control results for each tester strain exhibited a characteristic number of spontaneous revertants based on historical data collected at NAMSA. Each positive control mean exhibited at least a 3-fold increase over the respective negative control mean for each of the five tester strains (Table 12).

Table 11: Test Article to Negative Control Comparison

Tester Strain	Fold Over Negative Control - DMSO Test Article Extract*	Fold Over Negative Control - SC Test Article Extract*
TA98 without S9	0.9	1.3
TA98 with S9	0.9	1.0
TA100 without S9	1.0	0.7
TA100 with S9	1.0	0.9
TA1535 without S9	0.8	0.8
TA1535 with S9	1.1	1.1
TA1537 without S9	0.6	1.5
TA1537 with S9	0.9	0.8
WP2uvrA without S9	0.9	1.1
WP2uvrA with S9	0.8	1.0

*Value based on mean number of test extract revertants divided by the mean number of negative control revertants.

Values ≤ 1.0 represent no increase.

Table 12: Positive to Negative Control Comparison

Tester Strain + Positive Control	Fold Over DMSO Negative Control - Positive Controls*	Fold Over SC Negative Control - Positive Controls*
TA98 without S9 + 2-nitrofluorene	75.7	119.6
TA98 with S9 + benzo[a]pyrene	17.3	14.1
TA100 without S9 + sodium azide	16.8	12.6
TA100 with S9 + 2-aminoanthracene	13.5	12.1
TA1535 without S9 + sodium azide	232.9	169.9
TA1535 with S9 + 2-aminoanthracene	9.6	9.8
TA1537 without S9 + ICR-191	86.4	157.1
TA1537 with S9 + 2-aminoanthracene	26.8	19.5
WP2uvrA without S9 + methyl methanesulfonate	10.1	9.5
WP2uvrA with S9 + 2-aminoanthracene	7.2	7.0

* Value based on mean number of positive control revertants divided by the mean number of negative control revertants.

Values ≤ 1.0 represent no increase.

7. Conclusion

The DMSO and saline test article extracts were considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *Escherichia coli* tester strain WP2uvrA.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

8. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files in accordance with NAMSA SOPs.

9. References

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Appendix 1 - Tester Strain Revertants - Test Article Extract

Tester Strain	Background Lawn	Number of Revertants	Mean	Standard Deviation
TA98 DMSO test article extract without S9	Normal	11	18	6.5
	Normal	18		
	Normal	24		
TA98 DMSO test article extract with S9	Normal	15	19	4.0
	Normal	19		
	Normal	23		
TA100 DMSO test article extract without S9	Normal	114	117	14.7
	Normal	133		
	Normal	104		
TA100 DMSO test article extract with S9	Normal	136	138	5.7
	Normal	133		
	Normal	144		
TA1535 DMSO test article extract without S9	Normal	5	7	2.1
	Normal	9		
	Normal	8		
TA1535 DMSO test article extract with S9	Normal	13	13	1.0
	Normal	14		
	Normal	12		
TA1537 DMSO test article extract without S9	Slightly Reduced	3	4	0.6
	Slightly Reduced	4		
	Slightly Reduced	4		
TA1537 DMSO test article extract with S9	Slightly Reduced	4	5	1.2
	Normal	6		
	Normal	4		
WP2 ^{uvrA} DMSO test article extract without S9	Normal	45	59	12.8
	Normal	70		
	Normal	62		
WP2 ^{uvrA} DMSO test article extract with S9	Normal	71	62	8.2
	Normal	55		
	Normal	60		

Appendix 1 (continued) - Tester Strain Revertants - Test Article Extract

Tester Strain	Background Lawn	Number of Revertants	Mean	Standard Deviation
TA98 Saline test article extract without S9	Normal	16	16	1.5
	Normal	15		
	Normal	18		
TA98 Saline test article extract with S9	Normal	30	24	5.3
	Normal	22		
	Normal	20		
TA100 Saline test article extract without S9	Normal	120	108	15.9
	Normal	114		
	Normal	90		
TA100 Saline test article extract with S9	Normal	137	135	2.5
	Normal	135		
	Normal	132		
TA1535 Saline test article extract without S9	Normal	13	10	3.1
	Normal	7		
	Normal	9		
TA1535 Saline test article extract with S9	Normal	11	13	2.5
	Normal	16		
	Normal	13		
TA1537 Saline test article extract without S9	Normal	6	5	3.1
	Slightly Reduced	8		
	Normal	2		
TA1537 Saline test article extract with S9	Normal	7	6	2.6
	Normal	3		
	Slightly Reduced	8		
WP2 ^{uvrA} Saline test article extract without S9	Normal	80	73	7.6
	Normal	75		
	Normal	65		
WP2 ^{uvrA} Saline test article extract with S9	Normal	71	74	3.6
	Normal	78		
	Normal	73		

Appendix 2 - Tester Strain Revertants - Negative Control

Tester Strain	Background Lawn	Number of Revertants	Mean	Standard Deviation
TA98 DMSO negative control without S9	Normal	12	20	7.0
	Normal	23		
	Normal	25		
TA98 DMSO negative control with S9	Normal	27	20	6.1
	Normal	19		
	Normal	15		
TA100 DMSO negative control without S9	Normal	111	118	10.4
	Normal	130		
	Normal	113		
TA100 DMSO negative control with S9	Normal	139	140	11.0
	Normal	151		
	Normal	129		
TA1535 DMSO negative control without S9	Normal	8	9	1.7
	Normal	11		
	Normal	8		
TA1535 DMSO negative control with S9	Normal	13	12	2.1
	Normal	10		
	Normal	14		
TA1537 DMSO negative control without S9	Normal	6	7	4.0
	Normal	11		
	Normal	3		
TA1537 DMSO negative control with S9	Normal	11	5	4.9
	Normal	2		
	Normal	3		
WP2 ^{uvrA} DMSO negative control without S9	Normal	70	65	4.5
	Normal	61		
	Normal	65		
WP2 ^{uvrA} DMSO negative control with S9	Normal	75	73	4.4
	Normal	76		
	Normal	68		

Appendix 2 (continued) - Tester Strain Revertants - Negative Control

Tester Strain	Background Lawn	Number of Revertants	Mean	Standard Deviation
TA98 Saline negative control without S9	Normal	6	13	6.1
	Normal	18		
	Normal	14		
TA98 Saline negative control with S9	Normal	23	25	8.2
	Normal	18		
	Normal	34		
TA100 Saline negative control without S9	Normal	155	157	22.6
	Normal	181		
	Normal	136		
TA100 Saline negative control with S9	Normal	140	156	14.2
	Normal	167		
	Normal	161		
TA1535 Saline negative control without S9	Normal	18	12	5.1
	Normal	8		
	Normal	11		
TA1535 Saline negative control with S9	Normal	8	12	5.3
	Normal	18		
	Normal	10		
TA1537 Saline negative control without S9	Normal	6	4	2.1
	Normal	3		
	Normal	2		
TA1537 Saline negative control with S9	Normal	11	7	4.0
	Normal	3		
	Normal	8		
WP2 _{uvrA} Saline negative control without S9	Normal	65	70	4.2
	Normal	73		
	Normal	71		
WP2 _{uvrA} Saline negative control with S9	Normal	63	75	10.6
	Normal	79		
	Normal	83		

Appendix 3 - Tester Strain Revertants - Positive Controls

Tester Strain	Background Lawn	Number of Revertants	Mean	Standard Deviation
TA98 without S9 2-Nitrofluorene	Normal	2,064	1515	606.4
	Normal	1,616		
	Normal	864		
TA98 with S9 Benzo[a]pyrene	Normal	194	353	145.6
	Normal	480		
	Normal	384		
TA100 without S9 Sodium Azide	Normal	2,416	1984	530.4
	Normal	2,144		
	Normal	1,392		
TA100 with S9 2-Aminoanthracene	Normal	1,712	1883	151.2
	Normal	1,936		
	Normal	2,000		
TA1535 without S9 Sodium Azide	Normal	2,032	2096	340.5
	Normal	2,464		
	Normal	1,792		
TA1535 with S9 2-Aminoanthracene	Normal	123	118	7.8
	Normal	122		
	Normal	109		
TA1537 without S9 ICR-191	Normal	624	576	42.3
	Normal	560		
	Normal	544		
TA1537 with S9 2-Aminoanthracene	Normal	157	143	21.5
	Normal	153		
	Normal	118		
WP2uvrA without S9 Methyl methanesulfonate	Normal	896	661	218.4
	Normal	464		
	Normal	624		
WP2uvrA with S9 2-Aminoanthracene	Normal	608	523	88.1
	Normal	432		
	Normal	528		