charles river

FINAL REPORT

Test Facility Study No. 9601567

SM-102
Bacterial Reverse Mutation Test in Salmonella Typhimurium and Escherichia Coli

SPONSOR:
Moderna Therapeutics, Inc.
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Cambridge, MA 02139
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TEST FACILITY:
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12 January 2017

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COMPLIANCE STATEMENT

The study was performed in accordance with the OECD Principles of Good Laboratory Practice and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- Characterization of the Test Item was performed by the Sponsor subcontractor according to established SOPs, controls and approved test methodologies to ensure integrity and validity of the results generated; these analyses were not conducted in compliance with the GLP or GMP regulations.
- Stability testing of the supplied Test Item was not determined in this study. It will be performed by the Sponsor subcontractor at a laboratory that follows FDA Good Manufacturing Practice (GMP) regulations.

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Study Director are documented in the Study Records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

[redacted] Date: [redacted]

[redacted] MSc
Study Director

QUALITY ASSURANCE STATEMENT

Study Number: 9601567

This Study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows:

QA INSPECTION DATES

		Dates Findings Submitted to:	
Date(s) of Audit	Phase(s) Audited	Study Director	Study Director Management
12-Sep-2016	Final Study Plan	22-Sep-2016	22-Sep-2016
12-Sep-2016	Dose Preparation	22-Sep-2016	22-Sep-2016
22-Sep-2016	Study Plan Amendment 1	22-Sep-2016	22-Sep-2016
01-Nov-2016 - 02-Nov-2016	Data Review - Analytical Chemistry	04-Nov-2016	04-Nov-2016
01-Nov-2016 - 02-Nov-2016	Final Phase Report - Dose Formulation Analysis	04-Nov-2016	04-Nov-2016
08-Nov-2016 - 09-Nov-2016	Data Review - In Vitro Sciences	09-Nov-2016	09-Nov-2016
08-Nov-2016 - 09-Nov-2016	Final Report	09-Nov-2016	09-Nov-2016

In addition to the above-mentioned audits, process-based and/or routine facility inspections were also conducted during the course of this study. Inspection findings, if any, specific to this study were reported by Quality Assurance to the Study Director and Management and listed as a Phase Audit on this Quality Assurance Statement.

The Final Report has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

[redacted]
Quality Assurance Auditor

[redacted]
Date

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director	[redacted] MSc
Test Facility Management	[redacted] PhD, DABT

1.2. Individual Scientists (IS) at Test Facility

Dose Formulation Analysis	[redacted] BSc

2. SUMMARY

The objective of this study was to determine the potential genotoxicity of SM-102, using the bacterial reverse mutation test.

The experimental design was as follows:

Text Table 1
Plate Incorporation Assay

Dose No.	Formulation Conc. (µg/mL)	Dose Volume (µL/plate)	Final Conc. (µg/plate)	Number of Replicates		Number of Strains
				0S9	+S9	
Negative Control	-	100	-	3	3	5
1/ SM-102	19.0[b]	83.2	1.58	3	3	5
2/ SM-102	58.2[b]	85.9	5.0	3	3	5
3/ SM-102	184[b]	85.9	15.8	3	3	5
4/ SM-102	500[a]	100	50	3	3	5
5/ SM-102	1581[a]	100	158	3	3	5
6/ SM-102	5000[a]	100	500	3	3	5
7/ SM-102	15811[a]	100	1581	3	3	5
8/ SM-102	50000[a]	100	5000[c]	3	3	5
Positive controls	d	100	d	3	3	5

a Theoretical concentration; actual concentration may differ slightly due to the limitations of the instruments used.
b Measured concentration
c Test Item was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.
d Dose depends on the test organism, the positive controls and methodology used (see Section 4.5.1.2)

Salmonella typhimurium strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* were treated with the Test Item at a range of concentrations up to 5000 µg/plate (the standard limit dose for this assay), in the presence and absence of a supplemented rat liver fraction (S9 mix), using the plate incorporation version of the bacterial reverse mutation test.

Bacteria were incubated with standard positive controls, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix.

Incomplete, or absent, background lawns of non-revertant bacteria, or substantial reductions in revertant colony counts, were not obtained following exposure to SM-102, indicating that the Test Item was non-toxic to the bacteria at the levels tested. Precipitation was observed at concentrations ≥ 1581 µg/plate in the absence of S9 mix and at concentrations ≥ 500 µg/plate in the presence of S9 mix.

No substantial increases in revertant colony numbers were obtained with any of the tester strains, following exposure to SM-102 at any dose level, in either the presence or absence of S9 mix.

It is concluded that SM-102 did not show any evidence of genotoxic activity in this in vitro mutagenicity assay when tested in accordance with regulatory guidelines.

3. INTRODUCTION

The objective of this study was to determine the potential genotoxicity of SM-102, using the bacterial reverse mutation test.

The design of this study is based on OECD Guideline 471 and ICH Guideline S2(R1).

The Study Director signed the Study Plan on 08 Sep 2016, and dosing was initiated on 13 Sep 2016. The experimental start date was 12 Sep 2016, and the experimental completion date was 19 Sep 2016. The study was completed on the date of the Study Director approval of this report (refer to the appropriate signature page). The Study Plan, last Study Plan amendment, and deviations are presented in Appendix 1.

4. MATERIALS AND METHODS

4.1. Test and Reference Items

4.1.1. Test Item

Identification:	SM-102
Batch (Lot) No.:	RL-100-211-1
Retest Date:	27 Oct 2017
Purity:	95.72% (All concentrations and dose levels throughout this report were corrected for purity using a purity of 95.3%.)
Storage Conditions:	Kept in a freezer set to maintain -20°C

4.1.2. Reference Items

4.1.2.1. Negative Control

Identification:	Ethanol
Supplier:	Commercial Alcohols
Batch /Lot No.:	020612
Expiration Date:	June 2017
Storage Conditions:	Kept at ambient room temperature

4.1.2.2. Positive Controls

In the absence of S9 mix:

Identity:	Sodium azide (NaAz)
CAS number:	26628-22-8
Identity:	9-Aminoacridine hemihydrate (9AC)
CAS number:	65944-23-2

Identity:	2-Nitrofluorene (2NF)
CAS number:	607-57-8
Identity:	4-Nitroquinoline N-oxide (NQO)
CAS number:	56-57-5

In the presence of S9 mix:

Identity:	2-Aminoanthracene (2AA)
CAS number:	613-13-8
Identity:	Benzo[a]pyrene (BaP)
CAS number:	50-32-8

Full details of positive controls including supplier, lot number, storage, expiry date and formulation are retained as part of the Test Facility records. Copies of certificates of analysis are retained as raw data.

4.2. Test Item Characterization

The Sponsor provided to the Test Facility documentation of the identity, strength, purity, composition, and stability for the Test Item. A Certificate of Analysis was provided to the Test Facility and is presented in Appendix 2.

4.3. Analysis of Test Item

A Certificate of Analysis was provided by the Sponsor and is presented in Appendix 2.

4.4. Test Item Inventory and Disposition

Records of the receipt, distribution, and storage of Test Item were maintained with the study raw data. All unused Test Item was returned to the Sponsor following completion of the experimental phase of the study. Any remaining Reference Items (negative and positive controls) will be retained at the Test Facility or discarded upon expiry.

4.5. Dose Formulation and Analysis

4.5.1. Preparation of Reference Items

4.5.1.1. Preparation of Negative Control

An adequate amount of the Negative Control, ethanol, was dispensed into a vial for administration to control plates. The aliquot was stored in a refrigerator set to maintain 4°C until use. Any residual volumes were discarded before issuance of the Final Report.

4.5.1.2. Preparation of Positive Controls

The positive controls formulations were prepared up to 6 months prior to use. Adequate amounts were dispensed into vials, and stored in a freezer set to maintain -20°C, protected from light, until use. The aliquots were removed from the freezer and allowed to warm to ambient room temperature before dosing. Any residual volumes were discarded after completion of dosing.

Depending on the strains tested, the following positive controls were used:

Positive Controls for the Assay

Strain	S9	Positive Controls	Concentration µg/plate	Vehicle
TA1535, TA100	0	Sodium azide (NaAz)	0.5	Sterile water
TA1537		9-Aminoacridine hemihydrate (9AC)	50	DMSO
TA98		2-Nitrofluorene (2NF)	1	DMSO
WP2 *uvrA*		4-Nitroquinoline N-oxide (NQO)	0.5	DMSO
TA1535	+	2-Aminoanthracene (2AA)	5	DMSO
WP2 *uvrA*			20	
TA1537, TA98, TA100		Benzo[a]pyrene (BaP)	5	DMSO

4.5.2. Preparation of Test Item

The Test Item was prepared as a stock solution (50 mg/mL) in the chosen vehicle (ethanol) and all lower level formulations were made by serial dilution. The formulations were prepared 1 day prior to use and were stored in a refrigerator set to maintain 4°C until use. The formulations were removed from the refrigerator and allowed to warm to room temperature for at least 30 minutes before dosing. Any residual volumes were discarded before issuance of the Final Report.

4.5.3. Sample Collection and Analysis

The positive control formulations were not subjected to analysis for safety reasons and because the biological response of the test system is considered to be the best measure of the appropriateness of the formulations.

Dose formulations 1 to 8 were collected for concentration analysis only. Homogeneity, density and stability were not determined on these samples.

Samples to be analyzed were transferred at ambient room temperature, to the Analytical Chemistry department at the Test Facility on the date prepared. Any residual/retained analytical samples were discarded before issuance of the Final Report.

4.5.3.1. Analytical Method

Analyses were performed by HPLC, using a validated analytical procedure (Test Facility Study Number 1801841).

4.5.3.2. Concentration Analysis

Duplicate 1 mL samples for dose numbers 1 and 2 and duplicate 0.5 mL samples for dose numbers 3 to 8 were taken and sent to the analytical laboratory for analysis. Additional duplicate 1 mL samples for dose numbers 1 and 2 and duplicate 0.5 mL samples for dose numbers 3 to 8 were taken and retained at the Test Facility as backup samples. Concentration results were considered acceptable if mean sample concentration results were within ±10% of theoretical for the stock solution and ±15% of theoretical for lower level solutions. After acceptance of the analytical results, backup samples were discarded.

4.5.3.3. Stability Analysis

Stability analyses performed previously as part of a separate GLP-compliant study (Test Facility Study Number 1801841) demonstrated that the Test Item is stable in the vehicle when prepared and stored under the same conditions at concentrations bracketing those used in the present study.

4.6. Test System

The bacteria were originally supplied by Moltox, NC, USA. Each batch of frozen bacteria was tested for appropriate phenotype characteristics and spontaneous reversion rates; response to diagnostic mutagens is also routinely assessed. The following bacterial strains were employed:

S. typhimurium TA1535 *his*G46 *rfa ΔuvrB*

S. typhimurium TA1537 *his*C3076 *rfa ΔuvrB*

S. typhimurium TA98 *his*D3052 *rfa ΔuvrB* pKM101

S. typhimurium TA100 *his*G46 *rfa ΔuvrB* pKM101

E. coli WP2 *trp uvrA*

Fresh bacterial cultures were prepared so that they were in the late-log phase of growth at the time of use. The density of the cultures was confirmed to be $\geq 1000 \times 10^6$ bacteria/mL using a bacterial counting chamber before the cultures were used in the test.

4.6.1. Justification for Test System and Dose Level Selection

The bacterial reverse mutation test detects point mutations, which are the cause of many human genetic diseases and can play a role in tumor initiation and development.

Typically, the Test Item is dosed at a range of concentrations but is only assessed at the five highest levels below the toxic level or, if non-toxic, at five levels up to the standard limit of 5000 µg/plate.

4.7. S9 Mix

The S9 mix, used as a model of intact mammalian metabolism, was prepared on the day of use and contained 10% v/v S9 fraction (Aroclor 1254 induced male rat liver fraction supplied by Moltox) and the following sterile cofactors: 8 mM $MgCl_2$, 33 mM KCl, 100 mM sodium phosphate buffer pH 7.4, 5 mM glucose-6-phosphate and 4 mM NADP. The S9 mix was stored in a refrigerator set to maintain 4°C then held on ice during utilization. A copy of the manufacturer's quality control certificate for the S9 fraction is retained as raw data.

4.8. Sterility Check and Spontaneous Mutation Rates

The sterility of the Test Item formulation (high dose) was confirmed on the day of the test using appropriate preparations without bacteria.

The spontaneous mutation rates of the bacterial strains were assessed using concurrent control samples in which the bacteria were exposed to the Negative Control.

4.9. Plate Incorporation Method

A 0.5 mL aliquot of S9 mix (+S9) or phosphate buffer 0.2 M pH 7.4 (0S9) was combined with 0.1 mL bacterial culture in a sterile container. An aliquot of the test item (see Text Table 2 for test item dose volumes) or a 0.1 mL aliquot of reference item was added, then 2 mL of molten top agar supplemented with 0.05 mM biotin and minimal (0.05 mM) histidine and minimal (0.05 mM) tryptophan was added immediately afterward. The solution was mixed and overlaid onto a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose). After the overlay solidified, the plates were inverted and placed in an incubator set to maintain 37°C for 67 hours and 29 minutes.

4.10. Experimental Design

Text Table 2
Plate Incorporation Assay

Dose No.	Formulation Conc. (µg/mL)	Dose Volume (µL/plate)	Final Conc. (µg/plate)	Number of Replicates		Number of Strains
				0S9	+S9	
Negative Control	-	100	-	3	3	5
1/ SM-102	19.0[b]	83.2	1.58	3	3	5
2/ SM-102	58.2[b]	85.9	5.0	3	3	5
3/ SM-102	184[b]	85.9	15.8	3	3	5
4/ SM-102	500[a]	100	50	3	3	5
5/ SM-102	1581[a]	100	158	3	3	5
6/ SM-102	5000[a]	100	500	3	3	5
7/ SM-102	15811[a]	100	1581	3	3	5
8/ SM-102	50000[a]	100	5000[c]	3	3	5
Positive controls	d	100	d	3	3	5

a Theoretical concentrations; actual concentrations may differ slightly due to the limitations of the instruments used.
b Measured concentration
c Test Item was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.
d Dose depends on the test organism, the positive controls and methodology used (see Section 4.5.1.2)

5. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 3
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Sorcerer (Perceptive Instruments)	2.2	Revertant colony counts
Microsoft® Excel	2007	Descriptive statistics of revertant colony counts data.
Mesa Laboratories AmegaView CMS	v3.0 Build 1208.8	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO_2, as appropriate.
Johnson Controls Metasys	MVE 5.4 (M5)	Building Automation System. Control of HVAC and other building systems, as well as temperature/humidity control and trending in selected laboratories.

6. EVALUATION AND INTERPRETATION OF RESULTS

After the incubation period, the plates from bacterial reverse mutation test were examined visually; an inverted microscope was also employed to facilitate observations. Plates were evaluated for the quality of the background lawn and the number of revertant colonies. Revertant colony counts were collected and saved directly into an electronic database using an automated colony counter. Colony numbers were enumerated visually if precipitation or other artifacts interfered with the colony counter or at the discretion of the Study Director. The presence of visible precipitate was manually recorded in the raw data. Visual counts were performed for this study.

Toxic effects of the Test Item are normally indicated by the partial or complete absence of a background lawn (colony counts, if any, are not reported) or a substantial dose-related reduction in revertant colony counts compared with lower dose levels and concurrent Negative Control (*i.e.* fold response < 0.6) taking into account the laboratory historical control range.

The mean number of revertant colonies for all treatment groups was compared with those obtained for the concurrent Negative Control level.

6.1. Assay Acceptance Criteria:

Acceptable Negative Control: The number of revertants must be within or close the current historical negative control range of the laboratory.

Acceptable Positive Control: The mean value of a Positive Control for a tester strain must be compatible with those generated in the historical positive control data base and be substantially higher (≥ 2-fold) than the mean number of revertant colonies for its respective Negative Control.

Acceptable highest dose: Depending on the nature of the Test Item, it should reach the toxicity limit, but not exceed 5000 µg/plate, with at least 5 analyzable concentrations, if available.

In the event that the controls fall slightly outside the normal range (historical or Study Plan), the Study Director was allowed discretion to accept the results of the experiment as valid based on the biological significance.

6.2. Interpretation of Results

Biological relevance of the results was considered first.

Negative result (no evidence of genotoxicity) is concluded if there is no substantial increase (< 2-fold) in the number of colonies per plate in comparison to the concurrent Negative Control and the data is within or close to the 98% tolerance limit of the Negative Historical Control data. A negative result indicates that the Test Item is non-mutagenic in *S. typhimurium* and *E. coli*.

Positive result (evidence of genotoxicity) is concluded if there is a substantial increase (≥ 2-fold) in the number of colonies per plate in comparison to the concurrent Negative Control, the mean values are above the 98% tolerance limit of the Negative Historical Control data and a concentration-related increase over the exposure range tested is obtained. A positive result indicates that the Test Item induces point mutations in *S. typhimurium* and/or *E. coli*.

Equivocal result is concluded if no definite judgment can be made to fit the above criteria. An equivocal result indicates that a definitive conclusion cannot be made by performing the bacterial reverse mutation assay under the conditions described in the Study Plan. Alternate testing conditions (e.g., a narrower dose interval with the appropriate strain) may be used as an aid in evaluating the test results.

7. RETENTION OF RECORDS

All study-specific raw data, documentation, Study Plan, and final report from this study were archived at the Test Facility by no later than the date of final report issue. One year after issue of the unaudited Draft Report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

Electronic data generated by the Test Facility were archived as noted above, except the reporting files stored on SDMS, which were archived at the Charles River Laboratories facility location in Wilmington, MA.

8. RESULTS

8.1. Dose Formulation Analyses

Results of the dose formulation analyses are presented in Appendix 4.

The five highest formulation concentrations ranging from 500 to 50000 µg/mL met acceptance criteria, with chemical analysis indicating mean achieved concentrations within ±10% of the theoretical concentration for the stock solution and ±15% for lower level solutions. The lowest three formulation concentrations did not meet acceptance criteria (dose number 1 was +20% of nominal, dose number 2 was +16% of nominal and dose number 3 was +16% of nominal). Analyses of the retention samples were not performed due to the consistency of the results of the duplicate samples. Instead the results were accepted and the dose volumes of the three lowest test item concentrations to be tested were adjusted accordingly to obtain the concentrations specified in the study plan.

8.2. Bacterial Mutation Test

Results of the bacterial mutation test are presented in Table 1,Table 2 and Table 3; the mean and standard deviation values quoted have been rounded to the nearest whole number.

The absence of colonies on sterility check plate confirmed absence of microbial contamination (results not shown). The mean revertant colony counts for the Negative Control were within the laboratory historical control range (see Appendix 3 for individual colony count historical results). Appropriate positive controls (with S9 mix where required) induced increases in revertant colony numbers to at least twice the concurrent Negative Control levels with the appropriate bacterial strain, confirming sensitivity of the test system and activity of the S9 mix (see also Appendix 3 for positive controls historical results).

Incomplete, or absent, background lawns of non-revertant bacteria, or substantial reductions in revertant colony counts, were not obtained following exposure to SM-102, indicating that the Test Item was non-toxic to the bacteria at the levels tested. Precipitation was observed at concentrations ≥ 1581 µg/plate in the absence of S9 mix and at concentrations ≥ 500 µg/plate in the presence of S9 mix.

No substantial increases in revertant colony numbers were obtained with any of the tester strains, following exposure to SM-102 at any dose level, in either the presence or absence of S9 mix. Therefore, SM-102 was considered to be negative for the induction of mutagenicity in this in vitro assay.

9. CONCLUSION

It is concluded that SM-102 did not show any evidence of genotoxic activity in this in vitro mutagenicity assay when tested in accordance with regulatory guidelines.

Table 1 SM-102 - Plate Incorporation Assay in the Absence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants x_1	x_2	x_3	**mean**	SD	Plate observations * x_1	x_2	x_3	Fold response †
TA1535	Ethanol	0	12	11	20	**14**	5				1.0
	1.58	0	22	15	20	**19**	4				1.3
	5.0	0	18	19	8	**15**	6				1.0
	15.8	0	22	17	20	**20**	3				1.4
	50	0	22	23	19	**21**	2				1.5
	158	0	18	19	14	**17**	3				1.2
	500	0	20	17	19	**19**	2				1.3
	1581	0	9	15	15	**13**	3	ppt	ppt	ppt	0.9
	5000	0	18	16	13	**16**	3	ppt	ppt	ppt	1.1
TA1537	Ethanol	0	10	11	7	**9**	2				1.0
	1.58	0	11	15	10	**12**	3				1.3
	5.0	0	18	14	11	**14**	4				1.5
	15.8	0	11	9	14	**11**	3				1.2
	50	0	6	6	17	**10**	6				1.0
	158	0	14	14	3	**10**	6				1.1
	500	0	3	14	5	**7**	6				0.8
	1581	0	7	10	8	**8**	2	ppt	ppt	ppt	0.9
	5000	0	14	10	13	**12**	2	ppt	ppt	ppt	1.3
TA98	Ethanol	0	43	43	38	**41**	3				1.0
	1.58	0	38	29	43	**37**	7				0.9
	5.0	0	32	41	48	**40**	8				1.0
	15.8	0	34	27	38	**33**	6				0.8
	50	0	19	33	48	**33**	15				0.8
	158	0	33	31	28	**31**	3				0.7
	500	0	18	32	34	**28**	9				0.7
	1581	0	30	36	36	**34**	3	ppt	ppt	ppt	0.8
	5000	0	31	19	38	**29**	10	ppt	ppt	ppt	0.7

* Comments on the plate or background lawn: precipitate (ppt)
† Fold response in mean revertants compared to concurrent Negative Control.
SD Standard deviation

Table 1 SM-102 - Plate Incorporation Assay in the Absence of S9 Mix (Cont'd)

Strain	Conc. (µg/plate)	S9	Number of revertants x_1	x_2	x_3	**mean**	SD	Plate observations * x_1	x_2	x_3	Fold response †
TA100	Ethanol	0	134	124	142	**133**	9				1.0
	1.58	0	125	126	106	**119**	11				0.9
	5.0	0	126	126	117	**123**	5				0.9
	15.8	0	126	131	125	**127**	3				1.0
	50	0	108	127	109	**115**	11				0.9
	158	0	97	112	137	**115**	20				0.9
	500	0	106	85	106	**99**	12				0.7
	1581	0	122	113	118	**118**	5	ppt	ppt	ppt	0.9
	5000	0	124	116	86	**109**	20	ppt	ppt	ppt	0.8
WP2 *uvrA*	Ethanol	0	62	63	51	**59**	7				1.0
	1.58	0	55	29	56	**47**	15				0.8
	5.0	0	50	44	43	**46**	4				0.8
	15.8	0	64	50	46	**53**	9				0.9
	50	0	51	58	52	**54**	4				0.9
	158	0	41	41	43	**42**	1				0.7
	500	0	41	52	41	**45**	6				0.8
	1581	0	43	47	52	**47**	5	ppt	ppt	ppt	0.8
	5000	0	52	55	42	**50**	7	ppt	ppt	ppt	0.8

* Comments on the plate or background lawn: precipitate (ppt)
† Fold response in mean revertants compared to concurrent Negative Control.
SD Standard deviation

Table 2 SM-102 - Plate Incorporation Assay in the Presence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants x_1	x_2	x_3	**mean**	SD	Plate observations * x_1	x_2	x_3	Fold response †
TA1535	Ethanol	+	32	29	22	**28**	5				1.0
	1.58	+	14	23	20	**19**	5				0.7
	5.0	+	24	13	19	**19**	6				0.7
	15.8	+	28	25	22	**25**	3				0.9
	50	+	28	14	27	**23**	8				0.8
	158	+	18	19	27	**21**	5				0.8
	500	+	22	22	25	**23**	2	ppt	ppt	ppt	0.8
	1581	+	17	24	15	**19**	5	ppt	ppt	ppt	0.7
	5000	+	21	23	20	**21**	2	ppt	ppt	ppt	0.8
TA1537	Ethanol	+	15	15	17	**16**	1				1.0
	1.58	+	8	11	13	**11**	3				0.7
	5.0	+	14	10	6	**10**	4				0.6
	15.8	+	20	20	15	**18**	3				1.2
	50	+	18	17	10	**15**	4				1.0
	158	+	19	19	19	**19**	0				1.2
	500	+	19	14	15	**16**	3	ppt	ppt	ppt	1.0
	1581	+	8	12	13	**11**	3	ppt	ppt	ppt	0.7
	5000	+	17	10	18	**15**	4	ppt	ppt	ppt	1.0
TA98	Ethanol	+	41	59	60	**53**	11				1.0
	1.58	+	55	51	67	**58**	8				1.1
	5.0	+	61	38	38	**46**	13				0.9
	15.8	+	58	58	38	**51**	12				1.0
	50	+	51	39	56	**49**	9				0.9
	158	+	46	46	46	**46**	0				0.9
	500	+	53	57	44	**51**	7	ppt	ppt	ppt	1.0
	1581	+	67	52	68	**62**	9	ppt	ppt	ppt	1.2
	5000	+	49	50	54	**51**	3	ppt	ppt	ppt	1.0

* Comments on the plate or background lawn: precipitate (ppt)
† Fold response in mean revertants compared to concurrent Negative Control.
SD Standard deviation

Table 2 SM-102 - Plate Incorporation Assay in the Presence of S9 Mix (Cont'd)

Strain	Conc. (µg/plate)	S9	Number of revertants x_1	x_2	x_3	**mean**	SD	Plate observations * x_1	x_2	x_3	Fold response †
TA100	Ethanol	+	169	144	154	**156**	13				1.0
	1.58	+	150	123	153	**142**	17				0.9
	5.0	+	151	155	136	**147**	10				0.9
	15.8	+	174	172	160	**169**	8				1.1
	50	+	165	153	156	**158**	6				1.0
	158	+	135	131	131	**132**	2				0.9
	500	+	141	130	123	**131**	9	ppt	ppt	ppt	0.8
	1581	+	146	156	163	**155**	9	ppt	ppt	ppt	1.0
	5000	+	160	141	157	**153**	10	ppt	ppt	ppt	1.0
WP2 *uvrA*	Ethanol	+	49	55	62	**55**	7				1.0
	1.58	+	51	60	50	**54**	6				1.0
	5.0	+	43	62	65	**57**	12				1.0
	15.8	+	55	57	61	**58**	3				1.0
	50	+	53	58	60	**57**	4				1.0
	158	+	53	52	43	**49**	6				0.9
	500	+	39	36	53	**43**	9	ppt	ppt	ppt	0.8
	1581	+	63	48	44	**52**	10	ppt	ppt	ppt	0.9
	5000	+	47	38	38	**41**	5	ppt	ppt	ppt	0.7

* Comments on the plate or background lawn: precipitate (ppt)
† Fold response in mean revertants compared to concurrent Negative Control.
SD Standard deviation

Table 3 Positive Controls for the Plate Incorporation Assay

Strain	Treatment	Conc. (µg/plate)	S9	Number of revertants					Fold response †
				x_1	x_2	x_3	**mean**	SD	
TA1535	NaAz	0.5	0	398	409	395	**401**	7	28
TA1537	9AC	50	0	357	401	423	**394**	34	42
TA98	2NF	1	0	195	228	173	**199**	28	4.8
TA100	NaAz	0.5	0	524	518	500	**514**	12	3.9
WP2 *uvrA*	NQO	0.5	0	191	197	222	**203**	16	3.5
TA1535	2AA	5	+	252	240	263	**252**	12	9.1
TA1537	BaP	5	+	128	84	112	**108**	22	6.9
TA98	BaP	5	+	344	364	361	**356**	11	6.7
TA100	BaP	5	+	942	969	963	**958**	14	6.2
WP2 *uvrA*	2AA	20	+	202	190	216	**203**	13	3.7

† Fold response in mean revertants compared to concurrent Negative Control
SD Standard deviation