

AUDITED DRAFT REPORT

Study Title

**Bacterial Reverse Mutation Assay**

Test Article

1-bromopropane

Author

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Draft Report Date

21 August 2014

Testing Facility

BioReliance  
9630 Medical Center Drive  
Rockville, MD 20850

BioReliance Study Number

AD58PU.503001.BTL

Sponsor

Albemarle Corporation  
451 Florida Street  
Baton Rouge, LA 70801

## STATEMENT OF COMPLIANCE

Study No. AD58PU.503001.BTL was conducted in compliance with the US FDA Good Laboratory Practices 21 CFR Part 58 and the OECD Principles of Good Laboratory Practice (C(97)186/Final) in all material aspects with the following exception:

1. Test article dose formulations have been submitted for analysis, but a final GLP-compliant analytical report has not yet been completed.

Study Director Impact Statement: An assessment as to any impact on the study will be made once the report has been finalized.

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Valentine O. Wagner, III, M.S.  
Study Director

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Date

## **QUALITY ASSURANCE STATEMENT**

## STUDY INFORMATION

Sponsor: **Albemarle Corporation**  
**451 Florida Street**  
**Baton Rouge, LA 70801-1765**

Sponsor's Authorized Representative: **Carr Smith**

Testing Facility: **BioReliance**  
**9630 Medical Center Drive**  
**Rockville, MD 20850**

Test Article I.D.: **1-bromopropane**

Synonym: **N-Propyl Bromide**

Test Article Lot No.: **100000139331**

Test Article CAS No.: **106-94-5**

Test Article Molecular Weight: **123 g/mol**

BioReliance Study No.: **AD58PU.503001.BTL**

Test Article Description: **Clear colorless liquid**

Storage Conditions: **Room temperature, protected from light**

Test Article Receipt Date: **20 August 2013**

Study Initiation Date: **11 June 2014**

Experimental Starting Date (first day of data collection): **18 June 2014**

Experimental Start Date (first day test article administered to test system): **18 June 2014**

Experimental Completion Date: **30 July 2014**

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## SUMMARY

The test article, 1-bromopropane, was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvrA* in the presence or absence of Aroclor-induced rat liver S9. The assay was performed in two phases using the preincubation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article.

Ethanol (EtOH) was selected as the solvent of choice based on information provided by the Sponsor, solubility of the test article and compatibility with the target cells. The test article formed a clear solution in EtOH at approximately 500 mg/mL, the maximum concentration tested in the solubility test conducted at BioReliance.

In the initial toxicity-mutation assay, the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 200 mg/mL and a 25 µL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. No precipitate was observed. Toxicity was observed at 5000 µg per plate with all *Salmonella* tester strains. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 µg per plate. No precipitate was observed. Toxicity was observed at 5000 µg per plate with all *Salmonella* tester strains. However, due to technical errors, the entire assay was repeated.

In the retest of the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 3000 or at 5000 µg per plate with all tester strains.

Under the conditions of this study, test article 1-bromopropane was concluded to be negative in the Bacterial Reverse Mutation Assay.

## PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvrA* in the presence and absence of Aroclor-induced rat liver S9. A copy of the Historical Negative and Positive Control Values is included in [Appendix I](#). Copies of the study protocol and amendment are included in [Appendix II](#).

This study was conducted in compliance with the testing guidelines of the [OECD \(1997\)](#)

## CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, 1-bromopropane, was received by BioReliance on 20 August 2013 and was assigned the code number AD58PU. Upon receipt, the test article was stored at room temperature, protected from light.

The Sponsor has determined the identity, strength, purity and composition or other characteristics to define the test article and the stability of the test article. A copy of the Certificate of Analysis is included in [Appendix III](#). Based on the expiration date provided in the Certificate of Analysis, the test article was considered stable through 12 February 2015.

The vehicle used to deliver 1-bromopropane to the test system was EtOH (CAS No. 64-17-5, Lot Nos. SHBD7521V and SHBF2299V, Purity: 99.98%, Exp. Dates: October 2016 and April 2017, respectively), obtained from Sigma-Aldrich. Test article dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light. Test article dilutions were prepared in screw-capped tubes with minimal headspace in the retest of the confirmatory mutagenicity assay (see [Deviations](#)).

Dosing formulation samples were collected from each assay as follows:

- 2 x 3.5 mL from the high dose level and the vehicle and 2 x 17.0 mL from the low dose level in the initial toxicity-mutation assay
- 2 x 3.0 mL from the high dose level and the vehicle and 2 x 6.0 mL from the low dose level in the confirmatory mutagenicity assays

Additionally, treatment samples were collected from the vehicle, least and most concentrated test article preincubation tubes at the beginning and end of the preincubation period in each assay as follows:

- 4 x 0.525 mL from the high dose, low dose and vehicle preincubation tubes

All samples were submitted to the analytical chemistry laboratory at BioReliance for analysis. Unused samples will be discarded upon acceptance of the analytical results by the Study Director. A copy of the analytical report will be included in [Appendix IV](#).

The negative and positive control articles have been characterized as per the Certificates of Analysis on file with the testing facility. The stability of the negative and positive control articles and their mixtures was demonstrated by acceptable results that met the criteria for a valid test.

Positive controls plated concurrently with each assay are listed in the following table. All positive controls were diluted in dimethyl sulfoxide (DMSO) except for sodium azide, which was diluted in sterile water for injection-quality, cell culture grade water (hereafter referred to as sterile water). All subdivided solutions of positive control were stored at -10 to -30°C.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)		
TA98, TA1535	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. STBB1901V Exp. Date 31-Oct-2014 CAS No. 613-13-8 Purity 97.5%	1.0		
TA100, TA1537			2.0		
WP2 <i>uvrA</i>			15		
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. S43858V Exp. Date 31-Mar-2016 CAS No. 607-57-8 Purity 99.4%	1.0		
TA100, TA1535			sodium azide (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBH5113V Exp. Date 30-Jun-2016 CAS No. 26628-22-8 Purity 99.6%	1.0	
TA1537				9-aminoacridine (Sigma Aldrich Chemical Co., Inc.) Lot No. 09820CEV Exp. Date 31-Mar-2016 CAS No. 52417-22-8 Purity 99.4%	75
WP2 <i>uvrA</i>					methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBJ8702V and MKBG0368V Exp. Date 30-Apr-2016 and 31-Oct-2014 CAS No. 66-27-3 Purity 99.9%

To confirm the sterility of the test article and the vehicle, all test article dose levels and the vehicle used in each assay were plated on selective agar with an aliquot volume equal to that used in the assay. These plates were incubated under the same conditions as the assay.

## MATERIALS AND METHODS

### Test System

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by [Ames et al. \(1975\)](#) and *Escherichia coli* WP2 *uvrA* as described by [Green and Muriel \(1976\)](#). *Salmonella* tester strains were from Dr. Bruce Ames' Master cultures, *E. coli* tester strains were from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester strain TA1535 is reverted by mutagens that cause basepair substitutions. Tester strain TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations. Specificity of the reversion mechanism in *E. coli* is sensitive to base-pair substitution mutations, rather than frameshift mutations ([Green and Muriel, 1976](#)).

Overnight cultures were prepared by inoculating from the appropriate frozen permanent stock into a vessel, containing 30 to 50 mL of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a shaker/incubator programmed to begin shaking at 125 to 175 rpm and incubating at 37±2°C for approximately 12 hours before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of greater than or equal to 0.3x10<sup>9</sup> cells per milliliter. The actual titers were determined by viable count assays on nutrient agar plates.

### Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats that were injected intraperitoneally with Aroclor™ 1254 (200 mg/mL in corn oil) at a dose of 500 mg/kg, five days before sacrifice. The S9 (Lot Nos. 3267 and 3283, Exp. Dates: 29 April 2016 and 28 May 2016, respectively) was purchased commercially from Moltox (Boone, NC). Upon arrival at BioReliance, the S9 was stored at -60°C or colder until used. Each bulk preparation of S9 was assayed for its ability to metabolize benzo(a)pyrene and 2-aminoanthracene to forms mutagenic to *Salmonella typhimurium* TA100.

The S9 mix was prepared on the day of use as indicated below:

Component	Final Concentration
β-nicotinamide-adenine dinucleotide phosphate	4 mM
Glucose-6-phosphate	5 mM
Potassium chloride	33 mM
Magnesium chloride	8 mM
Phosphate Buffer (pH 7.4)	100 mM
S9 homogenate	10% (v/v)

The Sham S9 mixture (Sham mix), containing 100 mM phosphate buffer at pH 7.4, was also prepared on the day of use. To confirm the sterility of the S9 and Sham mixes, a 0.5 mL aliquot of each was plated on selective agar.

### **Solubility Test**

A solubility test was conducted using ethanol to determine the highest soluble or workable stock concentration up to 500 mg/mL.

### **Initial Toxicity-Mutation Assay**

The initial toxicity-mutation assay was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. Vehicle control, positive controls and eight dose levels of the test article were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvrA* on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9.

### **Confirmatory Mutagenicity Assay**

The confirmatory mutagenicity assay was used to evaluate and confirm the mutagenic potential of the test article. Seven dose levels of test article along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvrA* on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test article, vehicle control and positive controls were plated in triplicate.

### **Plating and Scoring Procedures**

The test system was exposed to the test article via the preincubation methodology described by [Yahagi \*et al.\* \(1977\)](#).

On the day of its use, minimal top agar, containing 0.8 % agar (W/V) and 0.5 % NaCl (W/V), was melted and supplemented with L-histidine, D-biotin and L-tryptophan solution to a final concentration of 50 µM each. Top agar not used with S9 or Sham mix was supplemented with 25 mL of sterile water for each 100 mL of minimal top agar. Bottom agar was Vogel-Bonner minimal medium E ([Vogel and Bonner, 1956](#)) containing 1.5 % (W/V) agar. Nutrient bottom agar was Vogel-Bonner minimal medium E containing 1.5 % (W/V) agar and supplemented with 2.5 % (W/V) Oxoid Nutrient Broth No. 2 (dry powder). Nutrient Broth was Vogel-Bonner salt solution supplemented with 2.5 % (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

Each plate was labeled by the BioReliance study number and a code system to designate the treatment condition, dose level and test phase, as described in detail in BioReliance's Standard Operating Procedures.

One-half (0.5) milliliter of S9 or sham mix, 100 µL of tester strain (cells seeded) and 25 µL of vehicle or test article dilution were added to 13 X 100 mm glass culture tubes pre-heated to 37±2°C. When plating the positive controls, the test article aliquot was replaced by a 50 µL aliquot of appropriate positive control. Tubes receiving test article were capped (screw caps)

during the preincubation period. After vortexing, these mixtures were incubated with shaking for 90±2 minutes at 37±2°C. Following the preincubation, 2.0 mL of selective top agar was added to each tube and the mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay had solidified, the plates receiving test article were inverted and placed in an appropriate number of 9- or 10-liter desiccators by dose level and incubated for 48 to 72 hours at 37±2°C. Plates receiving an aliquot of the vehicle or positive control articles were inverted after the overlay had solidified and also incubated for 48 to 72 hours at 37±2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the vehicle control plate using the codes shown in the following table.

Code	Description	Characteristics
1 or no code	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies compared to the vehicle control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn over more than or equal to 90% of the plate.
6	Obscured by Precipitate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article precipitate.
NP	Non-Interfering Precipitate	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 10% of the revertant colony count (e.g., less than 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., more than 3 particles on a plate with 30 revertants). These plates are counted manually.

Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

## Evaluation of Results

For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated and are reported.

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article.

Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response was evaluated as negative, if it was neither positive nor equivocal.

## Criteria for a Valid Test

The following criteria must be met for each assay to be considered valid. All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvrB* gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvrA* cultures must demonstrate the deletion in the *uvrA* gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvrA*, 10 - 60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to  $0.3 \times 10^9$  cells/mL. The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

## Automated Data Collection Systems

The primary computer or electronic systems used for the collection of data or analysis included but were not limited to the following:

Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments), LIMS System (BioReliance), Excel 2007 (Microsoft Corporation), BRIQS (BioReliance) and Kaye Lab Watch Monitoring System (Kaye GE).

## **Archives**

All raw data, the protocol, pertinent study email correspondence and all reports for procedures performed at BioReliance will be maintained in the archives at BioReliance, Rockville, MD for at least five years. At that time, the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials will first be copied and the copy will be retained by the BioReliance archives in accordance with the applicable SOPs. The raw data, reports and other documents generated at locations other than BioReliance will be archived by the test site.

## **Deviations**

The following deviations occurred during the conduct of this study:

Event No. 186462: Per a directive from the Study Director, the test article dilutions were to be prepared in screw-capped tubes with minimal headspace in the confirmatory mutagenicity assays to keep evaporation of the test article to a minimum. However, the use of screw-capped tubes was not documented for dilution preparation in the confirmatory mutagenicity assay. The results from this trial were consistent with those from the initial toxicity-mutation assay, but due to this documentation error and the additional deviation indicated below, the entire assay was retested. Therefore, the Study Director has concluded that this had no adverse impact on the integrity of the data or the validity of the study conclusion.

Event No. 186474: In the confirmatory mutagenicity assay, the amount of Sham mix needed to dose the assay, prepare the treatment tubes for analysis and perform the sterility plating was 80.5 mL, but only 80.0 mL was prepared. The results from this trial were consistent with those from the initial toxicity-mutation assay, but due to this calculation error and the additional deviation indicated above, the entire assay was retested. Therefore, the Study Director has concluded that this had no adverse impact on the integrity of the data or the validity of the study conclusion.

## RESULTS AND DISCUSSION

### Solubility Test

EtOH was selected as the solvent of choice based on information provided by the Sponsor, solubility of the test article and compatibility with the target cells. The test article formed a clear solution in EtOH at approximately 500 mg/mL, the maximum concentration tested in the solubility test conducted at BioReliance.

### Sterility Results

No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions or the S9 and Sham mixes.

### Tester Strain Titer Results

Experiment	Tester Strain				
	TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
	Titer Value (x 10 <sup>9</sup> cells per mL)				
B1	2.4	2.1	2.7	2.5	5.7
B2	2.9	1.4	4.1	4.3	5.4
B3	2.0	1.3	1.4	2.1	3.0

### Initial Toxicity-Mutation Assay

The results of the initial-toxicity mutation assay are presented in [Tables 1](#) and [2](#). These data were generated in Experiment B1.

In Experiment B1 (Initial Toxicity-Mutation Assay), the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 200 mg/mL and a 25 µL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. No precipitate was observed. Toxicity was observed at 5000 µg per plate with all *Salmonella* tester strains. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg per plate.

### Confirmatory Mutagenicity Assay

The results of the confirmatory mutagenicity assay are presented in [Tables 3](#) through [6](#). These data were generated in Experiments B2 and B3.

In Experiment B2 (Confirmatory Mutagenicity Assay), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The

dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 µg per plate. No precipitate was observed. Toxicity was observed at 5000 µg per plate with all *Salmonella* tester strains. However, the entire assay was repeated due to technical errors (see [Deviations](#)).

In Experiment B3 (Retest of the Confirmatory Mutagenicity Assay), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 3000 or at 5000 µg per plate with all tester strains.

A copy of the Common Technical Document Tables is included in [Appendix V](#).

### **Dosing Formulation Analysis**

Dosing formulation and treatment samples were delivered to the analytical chemistry laboratory at BioReliance for analysis. A copy of the report will be included in [Appendix IV](#). Upon finalization of the report, a summary of the results will be included in this section.

## **CONCLUSION**

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, 1-bromopropane did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9. Therefore, the test article was concluded to be negative in this assay.

## **REFERENCES**

Ames, B.N., J. McCann and E. Yamasaki (1975) Methods for Detecting Carcinogens and Mutagens with the *Salmonella*/Mammalian Microsome Mutagenicity Test, *Mutation Research*, 31:347-364.

Green, M.H.L. and W.J. Muriel (1976) Mutagen testing using trp+ reversion in *Escherichia coli*, *Mutation Research* 38:3-32.

Maron, D.M. and B.N. Ames (1983) Revised Methods for the *Salmonella* Mutagenicity Test, *Mutation Research*, 113:173-215.

OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, adopted July 21, 1997.

Vogel, H.J. and D.M. Bonner (1956) Acetylornithinase of *E. coli*: Partial Purification and Some Properties, *J. Biol. Chem.*, 218:97-106.

Yahagi, T., Nagao, M., Seino, Y., Matsushima, T., Sugimura, T. and Okada, M. (1977) Mutagenicities of N-nitrosamines on *Salmonella*, *Mutation Research*, 48:121-130.

## DATA TABLES

Table 1  
Initial Toxicity-Mutation Assay without S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B1  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/18/2014  
Evaluation Period: 6/23/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA98</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		1500 µg	17	1	1.2	16 <sup>A</sup> , 18 <sup>A</sup>
		500 µg	12	3	0.9	14 <sup>A</sup> , 10 <sup>A</sup>
		150 µg	22	4	1.6	24 <sup>A</sup> , 19 <sup>A</sup>
		50 µg	16	2	1.1	14 <sup>A</sup> , 17 <sup>A</sup>
		15 µg	15	3	1.1	17 <sup>A</sup> , 13 <sup>A</sup>
		5.0 µg	22	8	1.6	16 <sup>A</sup> , 27 <sup>A</sup>
		1.5 µg	15	1	1.1	15 <sup>A</sup> , 14 <sup>A</sup>
		<b>Ethanol</b>	25 µL	14	1	
<b>TA100</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 5, 0 <sup>M</sup> 5
		1500 µg	71	0	0.8	71 <sup>A</sup> , 71 <sup>A</sup>
		500 µg	106	1	1.2	105 <sup>A</sup> , 107 <sup>A</sup>
		150 µg	74	6	0.8	78 <sup>A</sup> , 70 <sup>A</sup>
		50 µg	96	1	1.1	97 <sup>A</sup> , 95 <sup>A</sup>
		15 µg	77	4	0.9	74 <sup>A</sup> , 80 <sup>A</sup>
		5.0 µg	84	13	1.0	93 <sup>A</sup> , 75 <sup>A</sup>
		1.5 µg	77	20	0.9	63 <sup>A</sup> , 91 <sup>A</sup>
		<b>Ethanol</b>	25 µL	88	11	
<b>TA1535</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		1500 µg	14	1	1.2	14 <sup>A</sup> , 13 <sup>A</sup>
		500 µg	9	1	0.8	10 <sup>A</sup> , 8 <sup>A</sup>
		150 µg	13	3	1.1	11 <sup>A</sup> , 15 <sup>A</sup>
		50 µg	11	4	0.9	8 <sup>A</sup> , 13 <sup>A</sup>
		15 µg	7	2	0.6	5 <sup>A</sup> , 8 <sup>A</sup>
		5.0 µg	9	3	0.8	7 <sup>A</sup> , 11 <sup>A</sup>
		1.5 µg	12	4	1.0	15 <sup>A</sup> , 9 <sup>A</sup>
		<b>Ethanol</b>	25 µL	12	1	

Key to Plate Postfix Codes

5 Absent background  
4 Extremely reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 1 cont.  
Initial Toxicity-Mutation Assay without S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B1  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/18/2014  
Evaluation Period: 6/23/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA1537</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		1500 µg	5	2	0.6	3 <sup>A</sup> , 6 <sup>A</sup>
		500 µg	6	1	0.7	7 <sup>A</sup> , 5 <sup>A</sup>
		150 µg	6	1	0.7	5 <sup>A</sup> , 7 <sup>A</sup>
		50 µg	8	1	0.9	7 <sup>A</sup> , 9 <sup>A</sup>
		15 µg	4	4	0.4	1 <sup>A</sup> , 6 <sup>A</sup>
		5.0 µg	7	1	0.8	7 <sup>A</sup> , 6 <sup>A</sup>
		1.5 µg	6	0	0.7	6 <sup>A</sup> , 6 <sup>A</sup>
		<b>Ethanol</b>	25 µL	9	3	
<b>WP2uvrA</b>	<b>1-bromopropane</b>	5000 µg	23	6	1.0	19 <sup>A</sup> 2, 27 <sup>A</sup> 2
		1500 µg	20	4	0.8	22 <sup>A</sup> , 17 <sup>A</sup>
		500 µg	26	5	1.1	29 <sup>A</sup> , 22 <sup>A</sup>
		150 µg	19	8	0.8	25 <sup>A</sup> , 13 <sup>A</sup>
		50 µg	24	1	1.0	24 <sup>A</sup> , 23 <sup>A</sup>
		15 µg	22	1	0.9	22 <sup>A</sup> , 21 <sup>A</sup>
		5.0 µg	20	1	0.8	21 <sup>A</sup> , 19 <sup>A</sup>
		1.5 µg	17	1	0.7	17 <sup>A</sup> , 16 <sup>A</sup>
		<b>Ethanol</b>	25 µL	24	1	
<b>TA98</b>	<b>2NF</b>	1.0 µg	761	65	54.4	715 <sup>A</sup> , 807 <sup>A</sup>
<b>TA100</b>	<b>SA</b>	1.0 µg	591	32	6.7	568 <sup>A</sup> , 613 <sup>A</sup>
<b>TA1535</b>	<b>SA</b>	1.0 µg	602	26	50.2	620 <sup>A</sup> , 583 <sup>A</sup>
<b>TA1537</b>	<b>9AAD</b>	75 µg	259	81	28.8	316 <sup>A</sup> , 201 <sup>A</sup>
<b>WP2uvrA</b>	<b>MMS</b>	1000 µg	563	33	23.5	586 <sup>A</sup> , 540 <sup>A</sup>

Key to Positive Controls

2NF 2-nitrofluorene  
SA sodium azide  
9AAD 9-Aminoacridine  
MMS methyl methanesulfonate

Key to Plate Postfix Codes

4 Extremely reduced background  
2 Slightly reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 2  
Initial Toxicity-Mutation Assay with S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B1  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/18/2014  
Evaluation Period: 6/23/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA98</b>	<b>1-bromopropane</b>	5000 µg	14	6	0.5	10 <sup>A</sup> 3, 18 <sup>A</sup> 3
		1500 µg	27	6	0.9	22 <sup>A</sup> , 31 <sup>A</sup>
		500 µg	29	5	1.0	25 <sup>A</sup> , 32 <sup>A</sup>
		150 µg	30	0	1.0	30 <sup>A</sup> , 30 <sup>A</sup>
		50 µg	20	6	0.7	24 <sup>A</sup> , 15 <sup>A</sup>
		15 µg	26	11	0.9	18 <sup>A</sup> , 34 <sup>A</sup>
		5.0 µg	24	2	0.8	25 <sup>A</sup> , 22 <sup>A</sup>
		1.5 µg	23	2	0.8	24 <sup>A</sup> , 21 <sup>A</sup>
		<b>Ethanol</b>	25 µL	29	7	
<b>TA100</b>	<b>1-bromopropane</b>	5000 µg	63	10	0.6	70 <sup>A</sup> 3, 56 <sup>A</sup> 3
		1500 µg	95	16	0.9	84 <sup>A</sup> , 106 <sup>A</sup>
		500 µg	103	8	1.0	97 <sup>A</sup> , 108 <sup>A</sup>
		150 µg	100	17	1.0	88 <sup>A</sup> , 112 <sup>A</sup>
		50 µg	89	1	0.9	88 <sup>A</sup> , 89 <sup>A</sup>
		15 µg	103	17	1.0	91 <sup>A</sup> , 115 <sup>A</sup>
		5.0 µg	93	6	0.9	97 <sup>A</sup> , 88 <sup>A</sup>
		1.5 µg	86	16	0.8	74 <sup>A</sup> , 97 <sup>A</sup>
		<b>Ethanol</b>	25 µL	104	0	
<b>TA1535</b>	<b>1-bromopropane</b>	5000 µg	10	2	0.9	8 <sup>A</sup> 3, 11 <sup>A</sup> 3
		1500 µg	13	8	1.2	18 <sup>A</sup> , 7 <sup>A</sup>
		500 µg	6	1	0.5	5 <sup>A</sup> , 6 <sup>A</sup>
		150 µg	13	0	1.2	13 <sup>A</sup> , 13 <sup>A</sup>
		50 µg	12	7	1.1	17 <sup>A</sup> , 7 <sup>A</sup>
		15 µg	15	1	1.4	14 <sup>A</sup> , 15 <sup>A</sup>
		5.0 µg	9	0	0.8	9 <sup>A</sup> , 9 <sup>A</sup>
		1.5 µg	11	6	1.0	15 <sup>A</sup> , 7 <sup>A</sup>
		<b>Ethanol</b>	25 µL	11	11	

Key to Plate Postfix Codes

3 Moderately reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 2 cont.  
Initial Toxicity-Mutation Assay with S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B1  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/18/2014  
Evaluation Period: 6/23/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA1537</b>	<b>1-bromopropane</b>	5000 µg	8	4	0.7	10 <sup>A</sup> 3, 5 <sup>A</sup> 3
		1500 µg	12	1	1.0	11 <sup>A</sup> , 13 <sup>A</sup>
		500 µg	9	3	0.8	11 <sup>A</sup> , 7 <sup>A</sup>
		150 µg	9	1	0.8	9 <sup>A</sup> , 8 <sup>A</sup>
		50 µg	15	1	1.3	15 <sup>A</sup> , 14 <sup>A</sup>
		15 µg	11	1	0.9	10 <sup>A</sup> , 11 <sup>A</sup>
		5.0 µg	8	1	0.7	8 <sup>A</sup> , 7 <sup>A</sup>
		1.5 µg	10	1	0.8	10 <sup>A</sup> , 9 <sup>A</sup>
		<b>Ethanol</b>	25 µL	12	4	
<b>WP2uvrA</b>	<b>1-bromopropane</b>	5000 µg	36	3	1.3	38 <sup>A</sup> , 34 <sup>A</sup>
		1500 µg	32	2	1.2	30 <sup>A</sup> , 33 <sup>A</sup>
		500 µg	34	13	1.3	43 <sup>A</sup> , 25 <sup>A</sup>
		150 µg	29	2	1.1	27 <sup>A</sup> , 30 <sup>A</sup>
		50 µg	28	1	1.0	29 <sup>A</sup> , 27 <sup>A</sup>
		15 µg	30	1	1.1	29 <sup>A</sup> , 31 <sup>A</sup>
		5.0 µg	23	1	0.9	22 <sup>A</sup> , 24 <sup>A</sup>
		1.5 µg	26	6	1.0	30 <sup>A</sup> , 22 <sup>A</sup>
		<b>Ethanol</b>	25 µL	27	3	
<b>TA98</b>	<b>2AA</b>	1.0 µg	435	5	15.0	431 <sup>A</sup> , 438 <sup>A</sup>
<b>TA100</b>	<b>2AA</b>	2.0 µg	504	60	4.8	546 <sup>A</sup> , 461 <sup>A</sup>
<b>TA1535</b>	<b>2AA</b>	1.0 µg	52	1	4.7	52 <sup>A</sup> , 51 <sup>A</sup>
<b>TA1537</b>	<b>2AA</b>	2.0 µg	47	1	3.9	47 <sup>A</sup> , 46 <sup>A</sup>
<b>WP2uvrA</b>	<b>2AA</b>	15 µg	310	2	11.5	311 <sup>A</sup> , 308 <sup>A</sup>

Key to Positive Controls

Key to Plate Postfix Codes

2AA 2-aminoanthracene

3 Moderately reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count

<sup>A</sup>: Automatic count

Table 3  
Confirmatory Mutagenicity Assay without S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B2  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/27/2014  
Evaluation Period: 6/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA98</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	19	7	0.8	25 <sup>M</sup> , 20 <sup>M</sup> , 12 <sup>M</sup>
		2000 µg	24	10	1.0	31 <sup>M</sup> , 29 <sup>M</sup> , 13 <sup>M</sup>
		1500 µg	27	1	1.1	26 <sup>M</sup> , 26 <sup>M</sup> , 28 <sup>M</sup>
		500 µg	23	7	1.0	31 <sup>M</sup> , 20 <sup>M</sup> , 17 <sup>M</sup>
		150 µg	19	3	0.8	16 <sup>M</sup> , 22 <sup>M</sup> , 19 <sup>M</sup>
		50 µg	21	3	0.9	18 <sup>M</sup> , 24 <sup>M</sup> , 20 <sup>M</sup>
		<b>Ethanol</b>	25 µL	24	3	
<b>TA100</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	67	17	0.7	86 <sup>A</sup> , 54 <sup>A</sup> , 60 <sup>A</sup>
		2000 µg	65	20	0.7	78 <sup>A</sup> , 42 <sup>A</sup> , 76 <sup>A</sup>
		1500 µg	72	22	0.7	47 <sup>A</sup> , 81 <sup>A</sup> , 88 <sup>A</sup>
		500 µg	80	16	0.8	65 <sup>A</sup> , 78 <sup>A</sup> , 96 <sup>A</sup>
		150 µg	91	8	0.9	93 <sup>A</sup> , 82 <sup>A</sup> , 97 <sup>A</sup>
		50 µg	90	8	0.9	91 <sup>A</sup> , 98 <sup>A</sup> , 82 <sup>A</sup>
		<b>Ethanol</b>	25 µL	97	12	
<b>TA1535</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	10	4	0.8	6 <sup>A</sup> , 10 <sup>A</sup> , 14 <sup>A</sup>
		2000 µg	8	4	0.6	13 <sup>A</sup> , 6 <sup>A</sup> , 6 <sup>A</sup>
		1500 µg	13	1	1.0	12 <sup>A</sup> , 13 <sup>A</sup> , 13 <sup>A</sup>
		500 µg	17	1	1.3	16 <sup>A</sup> , 18 <sup>A</sup> , 17 <sup>A</sup>
		150 µg	10	2	0.8	12 <sup>A</sup> , 10 <sup>A</sup> , 8 <sup>A</sup>
		50 µg	11	3	0.8	10 <sup>A</sup> , 8 <sup>A</sup> , 14 <sup>A</sup>
		<b>Ethanol</b>	25 µL	13	2	
<b>TA1537</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	7	3	0.9	5 <sup>A</sup> , 5 <sup>A</sup> , 11 <sup>A</sup>
		2000 µg	8	1	1.0	8 <sup>A</sup> , 7 <sup>A</sup> , 8 <sup>A</sup>
		1500 µg	6	6	0.8	13 <sup>A</sup> , 2 <sup>A</sup> , 2 <sup>A</sup>
		500 µg	8	4	1.0	5 <sup>A</sup> , 7 <sup>A</sup> , 12 <sup>A</sup>
		150 µg	11	3	1.4	13 <sup>A</sup> , 13 <sup>A</sup> , 7 <sup>A</sup>
		50 µg	11	3	1.4	11 <sup>A</sup> , 14 <sup>A</sup> , 8 <sup>A</sup>
		<b>Ethanol</b>	25 µL	8	3	

Key to Plate Postfix Codes

4 Extremely reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 3 cont.  
Confirmatory Mutagenicity Assay without S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B2  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/27/2014  
Evaluation Period: 6/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>WP2uvrA</b>	<b>1-bromopropane</b>	5000 µg	21	5	0.8	19 <sup>A</sup> 2, 17 <sup>A</sup> 2, 26 <sup>A</sup> 2
		3000 µg	21	5	0.8	24 <sup>A</sup> , 24 <sup>A</sup> , 16 <sup>A</sup>
		2000 µg	32	6	1.3	31 <sup>A</sup> , 38 <sup>A</sup> , 27 <sup>A</sup>
		1500 µg	33	3	1.3	35 <sup>A</sup> , 29 <sup>A</sup> , 35 <sup>A</sup>
		500 µg	30	13	1.2	27 <sup>A</sup> , 19 <sup>A</sup> , 45 <sup>A</sup>
		150 µg	32	10	1.3	22 <sup>A</sup> , 41 <sup>A</sup> , 32 <sup>A</sup>
		50 µg	27	3	1.1	23 <sup>A</sup> , 29 <sup>A</sup> , 29 <sup>A</sup>
	<b>Ethanol</b>	25 µL	25	5		31 <sup>A</sup> , 22 <sup>A</sup> , 23 <sup>A</sup>
<b>TA98</b>	<b>2NF</b>	1.0 µg	624	28	26.0	612 <sup>A</sup> , 656 <sup>A</sup> , 603 <sup>A</sup>
<b>TA100</b>	<b>SA</b>	1.0 µg	1120	55	11.5	1078 <sup>A</sup> , 1100 <sup>A</sup> , 1182 <sup>A</sup>
<b>TA1535</b>	<b>SA</b>	1.0 µg	836	37	64.3	793 <sup>A</sup> , 860 <sup>A</sup> , 855 <sup>A</sup>
<b>TA1537</b>	<b>9AAD</b>	75 µg	312	184	39.0	324 <sup>A</sup> , 490 <sup>A</sup> , 122 <sup>A</sup>
<b>WP2uvrA</b>	<b>MMS</b>	1000 µg	561	8	22.4	560 <sup>A</sup> , 569 <sup>A</sup> , 554 <sup>A</sup>
Key to Positive Controls			Key to Plate Postfix Codes			
2NF	2-nitrofluorene		2	Slightly reduced background		
SA	sodium azide					
9AAD	9-Aminoacridine					
MMS	methyl methanesulfonate					
Key to Automatic & Manual Count Flags						

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 4  
Confirmatory Mutagenicity Assay with S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B2  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/27/2014  
Evaluation Period: 6/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA98</b>	<b>1-bromopropane</b>	5000 µg	9	3	0.6	8 <sup>M</sup> 3, 6 <sup>M</sup> 3, 12 <sup>M</sup> 3
		3000 µg	14	2	1.0	14 <sup>M</sup> , 16 <sup>M</sup> , 13 <sup>M</sup>
		2000 µg	14	1	1.0	14 <sup>M</sup> , 13 <sup>M</sup> , 15 <sup>M</sup>
		1500 µg	14	3	1.0	11 <sup>M</sup> , 16 <sup>M</sup> , 14 <sup>M</sup>
		500 µg	10	1	0.7	10 <sup>M</sup> , 11 <sup>M</sup> , 10 <sup>M</sup>
		150 µg	14	3	1.0	16 <sup>M</sup> , 11 <sup>M</sup> , 16 <sup>M</sup>
		50 µg	13	5	0.9	9 <sup>M</sup> , 11 <sup>M</sup> , 19 <sup>M</sup>
	<b>Ethanol</b>	25 µL	14	4		18 <sup>M</sup> , 15 <sup>M</sup> , 10 <sup>M</sup>
<b>TA100</b>	<b>1-bromopropane</b>	5000 µg	59	5	0.6	62 <sup>A</sup> 3, NCN#, 55 <sup>A</sup> 3
		3000 µg	79	22	0.8	65 <sup>A</sup> , 104 <sup>A</sup> , 67 <sup>A</sup>
		2000 µg	81	26	0.8	65 <sup>A</sup> , 111 <sup>A</sup> , 67 <sup>A</sup>
		1500 µg	85	18	0.9	102 <sup>A</sup> , 87 <sup>A</sup> , 67 <sup>A</sup>
		500 µg	108	16	1.1	109 <sup>A</sup> , 123 <sup>A</sup> , 92 <sup>A</sup>
		150 µg	107	10	1.1	99 <sup>A</sup> , 118 <sup>A</sup> , 104 <sup>A</sup>
		50 µg	106	11	1.1	93 <sup>A</sup> , 110 <sup>A</sup> , 114 <sup>A</sup>
	<b>Ethanol</b>	25 µL	97	9		105 <sup>A</sup> , 87 <sup>A</sup> , 99 <sup>A</sup>
<b>TA1535</b>	<b>1-bromopropane</b>	5000 µg	17	1	1.3	17 <sup>A</sup> 3, 17 <sup>A</sup> 3, 18 <sup>A</sup> 3
		3000 µg	15	3	1.2	13 <sup>A</sup> , 14 <sup>A</sup> , 18 <sup>A</sup>
		2000 µg	21	3	1.6	23 <sup>A</sup> , 17 <sup>A</sup> , 23 <sup>A</sup>
		1500 µg	21	8	1.6	29 <sup>A</sup> , 14 <sup>A</sup> , 19 <sup>A</sup>
		500 µg	13	3	1.0	13 <sup>A</sup> , 11 <sup>A</sup> , 16 <sup>A</sup>
		150 µg	12	5	0.9	14 <sup>A</sup> , 6 <sup>A</sup> , 16 <sup>A</sup>
		50 µg	12	5	0.9	6 <sup>A</sup> , 14 <sup>A</sup> , 16 <sup>A</sup>
	<b>Ethanol</b>	25 µL	13	5		16 <sup>A</sup> , 16 <sup>A</sup> , 8 <sup>A</sup>
<b>TA1537</b>	<b>1-bromopropane</b>	5000 µg	12	3	0.9	CPN#, 14 <sup>A</sup> 3, 10 <sup>A</sup> 3
		3000 µg	6	1	0.5	7 <sup>A</sup> , 5 <sup>A</sup> , 7 <sup>A</sup>
		2000 µg	7	6	0.5	14 <sup>A</sup> , 2 <sup>A</sup> , 4 <sup>A</sup>
		1500 µg	8	2	0.6	6 <sup>A</sup> , 7 <sup>A</sup> , 10 <sup>A</sup>
		500 µg	6	1	0.5	5 <sup>A</sup> , 5 <sup>A</sup> , 7 <sup>A</sup>
		150 µg	7	4	0.5	7 <sup>A</sup> , 4 <sup>A</sup> , 11 <sup>A</sup>
		50 µg	5	5	0.4	5 <sup>A</sup> , 1 <sup>A</sup> , 10 <sup>A</sup>
	<b>Ethanol</b>	25 µL	13	3		16 <sup>A</sup> , 11 <sup>A</sup> , 12 <sup>A</sup>

Key to Plate Postfix Codes

3	Moderately reduced background
NC	No cells
N#	Not counted
CP	Contaminated plate

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 4 cont.  
Confirmatory Mutagenicity Assay with S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B2  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 6/27/2014  
Evaluation Period: 6/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>WP2uvrA</b>	<b>1-bromopropane</b>	5000 µg	31	2	1.0	33 <sup>A</sup> , 30 <sup>A</sup> , 29 <sup>A</sup>
		3000 µg	40	12	1.3	26 <sup>A</sup> , 47 <sup>A</sup> , 48 <sup>A</sup>
		2000 µg	39	11	1.3	44 <sup>A</sup> , 27 <sup>A</sup> , 47 <sup>A</sup>
		1500 µg	34	6	1.1	32 <sup>A</sup> , 41 <sup>A</sup> , 30 <sup>A</sup>
		500 µg	36	1	1.2	35 <sup>A</sup> , 37 <sup>A</sup> , 37 <sup>A</sup>
		150 µg	32	8	1.0	37 <sup>A</sup> , 36 <sup>A</sup> , 22 <sup>A</sup>
		50 µg	34	8	1.1	42 <sup>A</sup> , 27 <sup>A</sup> , 32 <sup>A</sup>
		25 µL	31	14		44 <sup>A</sup> , 31 <sup>A</sup> , 17 <sup>A</sup>
	<b>Ethanol</b>					
<b>TA98</b>	<b>2AA</b>	1.0 µg	519	49	37.1	571 <sup>A</sup> , 511 <sup>A</sup> , 474 <sup>A</sup>
<b>TA100</b>	<b>2AA</b>	2.0 µg	328	35	3.4	367 <sup>A</sup> , 320 <sup>A</sup> , 298 <sup>A</sup>
<b>TA1535</b>	<b>2AA</b>	1.0 µg	63	3	4.8	65 <sup>A</sup> , 59 <sup>A</sup> , 65 <sup>A</sup>
<b>TA1537</b>	<b>2AA</b>	2.0 µg	40	7	3.1	36 <sup>A</sup> , 48 <sup>A</sup> , 35 <sup>A</sup>
<b>WP2uvrA</b>	<b>2AA</b>	15 µg	334	83	10.8	424 <sup>A</sup> , 317 <sup>A</sup> , 260 <sup>A</sup>

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 5  
Retest of the Confirmatory Mutagenicity Assay without S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B3  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 7/24/2014  
Evaluation Period: 7/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA98</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	30	16	1.4	27 <sup>A</sup> , 16 <sup>A</sup> , 47 <sup>A</sup>
		1500 µg	35	6	1.6	31 <sup>A</sup> , 41 <sup>A</sup> , 32 <sup>A</sup>
		500 µg	36	1	1.6	35 <sup>A</sup> , 37 <sup>A</sup> , 37 <sup>A</sup>
		150 µg	37	7	1.7	37 <sup>A</sup> , 30 <sup>A</sup> , 44 <sup>A</sup>
		50 µg	39	5	1.8	44 <sup>A</sup> , 35 <sup>A</sup> , 39 <sup>A</sup>
		<b>Ethanol</b>	25 µL	22	5	
<b>TA100</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	75	3	0.7	73 <sup>A</sup> , 73 <sup>A</sup> , 79 <sup>A</sup>
		1500 µg	74	5	0.7	79 <sup>A</sup> , 75 <sup>A</sup> , 69 <sup>A</sup>
		500 µg	90	12	0.8	100 <sup>A</sup> , 76 <sup>A</sup> , 94 <sup>A</sup>
		150 µg	83	12	0.8	80 <sup>A</sup> , 73 <sup>A</sup> , 97 <sup>A</sup>
		50 µg	75	9	0.7	69 <sup>A</sup> , 72 <sup>A</sup> , 85 <sup>A</sup>
		<b>Ethanol</b>	25 µL	106	12	
<b>TA1535</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	13	4	0.9	17 <sup>A</sup> , 10 <sup>A</sup> , 12 <sup>A</sup>
		1500 µg	16	4	1.1	16 <sup>A</sup> , 19 <sup>A</sup> , 12 <sup>A</sup>
		500 µg	18	6	1.2	23 <sup>A</sup> , 20 <sup>A</sup> , 11 <sup>A</sup>
		150 µg	18	3	1.2	16 <sup>A</sup> , 16 <sup>A</sup> , 22 <sup>A</sup>
		50 µg	15	1	1.0	16 <sup>A</sup> , 14 <sup>A</sup> , 14 <sup>A</sup>
		<b>Ethanol</b>	25 µL	15	4	
<b>TA1537</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	3	1	0.5	3 <sup>M</sup> , 3 <sup>M</sup> , 4 <sup>M</sup>
		1500 µg	5	2	0.8	3 <sup>M</sup> , 5 <sup>M</sup> , 6 <sup>M</sup>
		500 µg	4	1	0.7	4 <sup>M</sup> , 4 <sup>M</sup> , 3 <sup>M</sup>
		150 µg	3	1	0.5	2 <sup>M</sup> , 4 <sup>M</sup> , 3 <sup>M</sup>
		50 µg	4	1	0.7	4 <sup>M</sup> , 4 <sup>M</sup> , 3 <sup>M</sup>
		<b>Ethanol</b>	25 µL	6	1	

Key to Plate Postfix Codes

4 Extremely reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 5 cont.  
Retest of the Confirmatory Mutagenicity Assay without S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B3  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 7/24/2014  
Evaluation Period: 7/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>WP2uvrA</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	33	13	1.0	48 <sup>A</sup> , 24 <sup>A</sup> , 26 <sup>A</sup>
		2000 µg	29	7	0.9	27 <sup>A</sup> , 24 <sup>A</sup> , 37 <sup>A</sup>
		1500 µg	24	2	0.8	24 <sup>A</sup> , 26 <sup>A</sup> , 22 <sup>A</sup>
		500 µg	32	6	1.0	25 <sup>A</sup> , 37 <sup>A</sup> , 35 <sup>A</sup>
		150 µg	27	6	0.8	29 <sup>A</sup> , 20 <sup>A</sup> , 32 <sup>A</sup>
		50 µg	26	5	0.8	22 <sup>A</sup> , CPN#, 29 <sup>A</sup>
	<b>Ethanol</b>	25 µL	32	2		35 <sup>A</sup> , 31 <sup>A</sup> , 31 <sup>A</sup>
<b>TA98</b>	<b>2NF</b>	1.0 µg	768	62	34.9	698 <sup>A</sup> , 816 <sup>A</sup> , 791 <sup>A</sup>
<b>TA100</b>	<b>SA</b>	1.0 µg	1057	60	10.0	989 <sup>A</sup> , 1099 <sup>A</sup> , 1084 <sup>A</sup>
<b>TA1535</b>	<b>SA</b>	1.0 µg	463	5	30.9	462 <sup>A</sup> , 468 <sup>A</sup> , 459 <sup>A</sup>
<b>TA1537</b>	<b>9AAD</b>	75 µg	115	2	19.2	114 <sup>A</sup> , 117 <sup>A</sup> , 114 <sup>A</sup>
<b>WP2uvrA</b>	<b>MMS</b>	1000 µg	614	58	19.2	548 <sup>A</sup> , 650 <sup>A</sup> , 645 <sup>A</sup>

Key to Positive Controls

2NF 2-nitrofluorene  
SA sodium azide  
9AAD 9-Aminoacridine  
MMS methyl methanesulfonate

Key to Plate Postfix Codes

4 Extremely reduced background  
CP Contaminated plate  
N# Not counted

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 6  
Retest of the Confirmatory Mutagenicity Assay with S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B3  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 7/24/2014  
Evaluation Period: 7/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>TA98</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	23	8	0.7	19 <sup>M</sup> , 32 <sup>M</sup> , 17 <sup>M</sup>
		1500 µg	25	3	0.8	29 <sup>M</sup> , 23 <sup>M</sup> , 24 <sup>M</sup>
		500 µg	25	4	0.8	28 <sup>M</sup> , 21 <sup>M</sup> , 27 <sup>M</sup>
		150 µg	24	3	0.8	21 <sup>M</sup> , 27 <sup>M</sup> , 25 <sup>M</sup>
		50 µg	25	5	0.8	22 <sup>M</sup> , 22 <sup>M</sup> , 31 <sup>M</sup>
		<b>Ethanol</b>	25 µL	32	6	
<b>TA100</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	68	6	0.7	62 <sup>A</sup> , 74 <sup>A</sup> , 67 <sup>A</sup>
		1500 µg	78	14	0.8	66 <sup>A</sup> , 94 <sup>A</sup> , 75 <sup>A</sup>
		500 µg	88	11	0.9	87 <sup>A</sup> , 99 <sup>A</sup> , 78 <sup>A</sup>
		150 µg	88	13	0.9	103 <sup>A</sup> , 80 <sup>A</sup> , 80 <sup>A</sup>
		50 µg	106	27	1.1	125 <sup>A</sup> , 87 <sup>A</sup> , CPN#
		<b>Ethanol</b>	25 µL	100	32	
<b>TA1535</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	20	3	0.6	20 <sup>A</sup> , 23 <sup>A</sup> , 17 <sup>A</sup>
		1500 µg	20	6	0.6	13 <sup>A</sup> , 24 <sup>A</sup> , 24 <sup>A</sup>
		500 µg	24	2	0.8	22 <sup>A</sup> , 25 <sup>A</sup> , 26 <sup>A</sup>
		150 µg	32	3	1.0	31 <sup>A</sup> , 35 <sup>A</sup> , 29 <sup>A</sup>
		50 µg	26	3	0.8	23 <sup>A</sup> , 29 <sup>A</sup> , 27 <sup>A</sup>
		<b>Ethanol</b>	25 µL	31	7	
<b>TA1537</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	7	2	1.4	5 <sup>M</sup> , 8 <sup>M</sup> , 7 <sup>M</sup>
		1500 µg	5	2	1.0	5 <sup>M</sup> , 6 <sup>M</sup> , 3 <sup>M</sup>
		500 µg	4	2	0.8	6 <sup>M</sup> , 3 <sup>M</sup> , 4 <sup>M</sup>
		150 µg	4	1	0.8	4 <sup>M</sup> , 5 <sup>M</sup> , 3 <sup>M</sup>
		50 µg	4	1	0.8	4 <sup>M</sup> , 5 <sup>M</sup> , 4 <sup>M</sup>
		<b>Ethanol</b>	25 µL	5	1	

Key to Plate Postfix Codes

4 Extremely reduced background  
CP Contaminated plate  
N# Not counted

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count      <sup>A</sup>: Automatic count

Table 6 cont.  
Retest of the Confirmatory Mutagenicity Assay with S9 activation

Study Number: AD58PU.503001.BTL  
Experiment: B3  
Exposure Method: Pre-incubation assay

Study Code: AD58PU  
Date Plated: 7/24/2014  
Evaluation Period: 7/30/2014

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
<b>WP2uvrA</b>	<b>1-bromopropane</b>	5000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		3000 µg	0	0	0.0	0 <sup>M</sup> 4, 0 <sup>M</sup> 4, 0 <sup>M</sup> 4
		2000 µg	31	10	0.8	25 <sup>A</sup> , 26 <sup>A</sup> , 42 <sup>A</sup>
		1500 µg	28	4	0.7	25 <sup>A</sup> , 32 <sup>A</sup> , 26 <sup>A</sup>
		500 µg	25	2	0.6	24 <sup>A</sup> , 23 <sup>A</sup> , 27 <sup>A</sup>
		150 µg	33	5	0.8	27 <sup>A</sup> , 36 <sup>A</sup> , 35 <sup>A</sup>
		50 µg	32	9	0.8	30 <sup>A</sup> , 24 <sup>A</sup> , 42 <sup>A</sup>
		<b>Ethanol</b>	25 µL	41	10	
<b>TA98</b>	<b>2AA</b>	1.0 µg	490	7	15.3	485 <sup>A</sup> , 486 <sup>A</sup> , 498 <sup>A</sup>
<b>TA100</b>	<b>2AA</b>	2.0 µg	554	53	5.5	562 <sup>A</sup> , 602 <sup>A</sup> , 497 <sup>A</sup>
<b>TA1535</b>	<b>2AA</b>	1.0 µg	105	12	3.4	93 <sup>A</sup> , 106 <sup>A</sup> , 117 <sup>A</sup>
<b>TA1537</b>	<b>2AA</b>	2.0 µg	87	16	17.4	72 <sup>A</sup> , 103 <sup>A</sup> , 85 <sup>A</sup>
<b>WP2uvrA</b>	<b>2AA</b>	15 µg	402	15	9.8	401 <sup>A</sup> , 418 <sup>A</sup> , 388 <sup>A</sup>

Key to Positive Controls

Key to Plate Postfix Codes

2AA 2-aminoanthracene

4 Extremely reduced background

Key to Automatic & Manual Count Flags

<sup>M</sup>: Manual count

<sup>A</sup>: Automatic count

**APPENDIX I: Historical Control Data**

Historical Negative and Positive Control Values  
2011 – 2013

revertants per plate

Strain	Control	Activation							
		None				Rat Liver			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA98	Neg	18	8	3	64	24	8	4	60
	Pos	271	214	34	2274	423	190	47	1711
TA100	Neg	98	18	50	251	110	23	55	247
	Pos	608	143	211	1393	730	260	247	2421
TA1535	Neg	11	4	1	43	12	4	1	35
	Pos	501	164	20	1593	103	92	20	1472
TA1537	Neg	7	4	0	28	8	4	0	28
	Pos	422	386	17	3711	59	56	10	850
WP2 <i>uvrA</i>	Neg	27	10	5	84	30	10	7	80
	Pos	380	160	42	1796	245	98	21	969

SD=standard deviation; Min=minimum value; Max=maximum value; Neg=negative control (including but not limited to deionized water, dimethyl sulfoxide, ethanol and acetone); Pos=positive control

## **APPENDIX II: Study Protocol**

PROTOCOL AMENDMENT 1

Sponsor: Albemarle Corporation

BioReliance Study No.: AD58PU.503001.BTL

Title: Bacterial Reverse Mutation Assay

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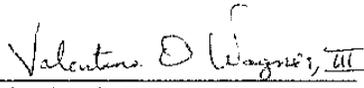
Effective: 18-Jun-2014

1. Page 9, Section 7.10, Treatment of Test System

Replace: "9-liter desiccators" With: "9-liter or 10-liter desiccators"

Reason: The 9-liter desiccators are no longer available and the newer ones in use are 10 liters.

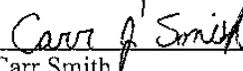
**Approvals:**

  
Valentine O. Wagner, III, MS  
BioReliance Study Director

18 Jun 2014  
Date

  
BioReliance Study Management

18 JUN 2014  
Date

  
Carr Smith  
Sponsor Representative

26 JUNE 2014  
Date

### Bacterial Reverse Mutation Assay

#### 1.0 PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test article by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvrA* in the presence and absence of S9 activation.

#### 2.0 SPONSOR

- 2.1 Sponsor Name: Albemarle Corporation
- 2.2 Address: 451 Florida Street  
Baton Rouge, LA 70801
- 2.3 Representative: Carr Smith  
Phone: 225-388-7545  
Email: carr.smith@albemarle.com

#### 3.0 TEST AND CONTROL ARTICLES

- 3.1 Test Article: 1-bromopropane
- Storage Temperature: Controlled room temperature
- Storage Parameters: Unless otherwise indicated, all test articles will be stored protected from light.
- Purity: An adjustment for purity or active ingredient will not be made unless indicated by the Sponsor.
- Molecular Weight: 123 g/mol
- 3.2 Controls: Negative: Test article vehicle  
Positive: 9-aminoacridine  
2-aminoanthracene  
methyl methanesulfonate  
2-nitrofluorene  
sodium azide

#### 3.3 Characterization and Stability of the Test Article and Test Article Mixtures

BioReliance will not perform analysis of the test article. The Sponsor will be directly responsible for determination and documentation of the analytical purity, composition and stability of the test article, and the stability and strength of the test article in the solvent (or vehicle). If there is no characterization and/or stability

analysis of the test article, the GLP compliance statement in the final report will cite these deficiencies as exceptions to the GLP regulations with which this study is compliant.

### 3.4 Characterization of Test Article Dose Formulations at BioReliance

The dose formulations will be analyzed by the analytical chemistry laboratory at BioReliance as outlined below and per BioReliance Standard Operating Procedure OPAC0385, Dose Formulation Analysis.

#### 3.4.1 Analytical Method Validation

The analytical method validated for BioReliance study number AD58PU.GTCHEM.BTL will be employed.

#### 3.4.2 Collection of Dose Formulation and Treatment Samples

Dosing sample analysis will be conducted on the day that the samples are prepared, unless stability information allows samples to be analyzed on a different day.

For each initial toxicity-mutation assay trial and each confirmatory mutagenicity assay trial, samples will be collected as follows.

- Vehicle, least and most concentrated test article formulations in ethanol. These samples are termed the formulation samples. If the low dose is below the experimental limit of quantitation determined during validation of the analytical method, then the dose with the lowest concentration that is above the limit of quantitation will be analyzed in place of the low dose.
- Vehicle, least and most concentrated test article preincubation tubes at the beginning and end of the preincubation period. These samples are termed the treatment samples.
- These samples will be dosed in at least **quadruplicate** with 0.5 mL sham mix and an appropriate aliquot of vehicle and test article at the lowest and highest analyzable concentrations. If the low dose is below the experimental limit of quantitation determined during validation of the analytical method, then the dose with the lowest concentration that is above the limit of quantitation will be analyzed in place of the low dose.
- If necessary, as noted by the analytical chemist, alternate collection parameters will be used for any of the above samples.
- Any residual samples will be discarded when permission to finalize is received from the Study Director and Sponsor.

All samples collected for analysis or as backups will be stored at -10 to -30°C.

#### 3.4.3 Acceptance Criteria

- Formulation samples in ethanol will be analyzed in duplicate and must be within 85.0% to 115.0% of their target concentrations and the % RSD of each concentration must be  $\leq 5.00\%$ .
- The formulation vehicle control sample will be analyzed only once, to confirm the absence of test article. For a vehicle sample to be reported as free of test article, the concentration of the test article in the vehicle formulation must be below the Limit of Detection of the analytical method.
- The treatment samples will be analyzed in duplicate and reported as found with no acceptance criteria applied.

In the event that a sample is outside of the acceptable specification range, the Study Director will justify the acceptability of the results or suggest re-analysis of the backup samples or retest the affected portion of the study.

#### 3.4.4 Stability

If the stability of the test article in the vehicle has already been determined and the target concentrations bracket the concentrations of the submitted samples then the stability analysis will not be performed and the study for which the stability was determined will be cited in the report. When stability has not been established over the appropriate range, it will be conducted by reanalyzing the highest and/or lowest from one set of dosing formulations after at least 3 hours storage at room temperature (15 to 30°C). The stability analysis will be conducted identically to the dosing formulation analysis. For suspensions one sample will be analyzed from each level of the formulation. The concentrations obtained must be within 90 to 110% of the original concentrations to be considered stable. If the label concentration of any submitted sample is outside of the established stability concentration range, the stability analysis will be repeated to extend the range. Alternatively, the chemistry laboratory may establish stability on samples prepared in their laboratory at the appropriate concentration(s).

#### 3.4.5 Data Collection, Storage and Analysis

Data will be collected using Windows based desktop PCs running Agilent ChemStation with the ChemStation security pack installed. An automatic process will immediately transfer all data generated to an Oracle database by ChemStation. The database is controlled by ChemStore C/S and has a

full audit trail tracking system. This data will be maintained electronically per Standard Operating Procedure ODIS3100, Policy for Data Backup/Recovery/Retention. The exact revisions of the software used will be documented in the raw data and included in the final report. Data analysis will be performed using a combination of Agilent ChemStation (Revision to be recorded in the raw data and reported in the Final Report), Chem2XL (version 3.3.1) and Microsoft Excel as needed.

3.4.6 Compliance

The work performed in conjunction with the dose formulation analyses will be conducted in compliance with the study protocol and protocol amendments, appropriate standard operating procedures of the analytical laboratory and GLPs (listed in section 12 of this protocol). All deviations and QA findings from the analytical portion of this study will be reported to the Study Director and BioReliance Management.

3.4.7 Reporting

A draft report that summarizes the methods, analysis, and results carried out by the Analytical Chemistry laboratory will be provided to the Study Monitor/Representative and Study Director. After acceptance of the results, the final report will be prepared and submitted to the Study Director for inclusion in the draft main study report, as an appendix.

3.5 Test Article Retention Sample

Since the in-life portion of this study is less than four weeks in duration, BioReliance will not retain a reserve sample of the test article.

3.6 Residual Test Article and Dosing Preparations

Dosing preparations, excluding those saved for concentration or homogeneity analysis (if any), will be disposed of following administration to the test system. Following finalization of the report, residual test article will be discarded unless otherwise indicated by the Sponsor.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1 Name: Toxicology Testing Facility  
BioReliance

4.2 Address: 9630 Medical Center Drive  
Rockville, MD 20850

4.3 Study Director: Valentine O. Wagner III, M.S.  
 Phone: 301-610-2152  
 Fax: 301-738-2362  
 Email: skip.wagner@bioreliance.com

4.4 Analytical Chemist (Dose Formulation Analysis)  
 Philip Atkins, MChem  
 9630 or 9610 Medical Center Drive  
 Rockville, MD 20850  
 Phone: 301-610-2114  
 Fax: 301-738-2362  
 Email: philip.atkins@bioreliance.com

4.5 Quality Assurance Unit of BioReliance (Lead QA):  
 Karen Westray  
 QA Manager, Toxicology  
 Phone: 301-610-2856  
 Fax: 301-738-2362  
 Email: Karen.westray@bioreliance.com

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 18 June 2014

5.2 Proposed Experimental Completion Date: 17 July 2014

5.3 Proposed Report Date: 31 July 2014

6.0 TEST SYSTEM

The tester strains will include the *S. typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al.* (1975) and the *E. coli* tester strain WP2 *uvrA* as described by Green and Muriel (1976).

Histidine Mutation			Tryptophan Mutation	Additional Mutations		
<i>hisG46</i>	<i>hisC3076</i>	<i>hisD3052</i>	<i>trpE</i>	LPS	Repair	R-factor
TA1535	TA1537	-	-	<i>rfa</i>	$\Delta$ <i>uvrB</i>	-
TA100	-	TA98	-	<i>rfa</i>	$\Delta$ <i>uvrB</i>	+R
-	-	-	WP2 <i>uvrA</i>	-	$\Delta$ <i>uvrA</i>	-

Each *S. typhimurium* tester strain contains, in addition to a mutation in the histidine operon, additional mutations that enhance sensitivity to some mutagens. The *rfa* mutation results in a cell wall deficiency that increases the permeability of the cell to certain classes of chemicals such as those containing large ring systems that would otherwise be excluded. The deletion in the *uvrB* gene results in a deficient DNA excision-repair system. Tester strains TA98 and TA100 also contain the pKM101

plasmid (carrying the R-factor). It has been suggested that the plasmid increases sensitivity to mutagens by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. TA100 is reverted by both frameshift and base substitution mutagens and TA1535 is reverted only by mutagens that cause base substitutions.

The *E. coli* tester strain has an AT base pair at the critical mutation site within the *trpE* gene (Wilcox *et al.*, 1990). Tester strain WP2 *uvrA* has a deletion in the *uvrA* gene resulting in a deficient DNA excision-repair system. Tryptophan revertants can arise due to a base change at the originally mutated site or by a base change elsewhere in the chromosome causing the original mutation to be suppressed. Thus, the specificity of the reversion mechanism is sensitive to base-pair substitution mutations (Green and Muriel, 1976).

The *S. typhimurium* tester strains were from Dr. Bruce Ames, University of California, Berkeley. The *E. coli* tester strain was from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (United Kingdom). The tester strains may also be obtained from Molecular Toxicology Inc. (Moltox).

## 7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

### 7.1 Solubility

The test article was found to be soluble in ethanol at 200 mg/mL and ethanol will be used as the test article vehicle.

### 7.2 Initial Toxicity-Mutation Assay

Selection of dose levels for the confirmatory mutagenicity assay will be based upon the toxicity and precipitation profile of the test article assessed in an initial toxicity-mutation assay. The test article will be tested at a minimum of eight dose levels along with appropriate negative and positive controls with tester strains TA98, TA100, TA1535, TA1537 and WP2 *uvrA* with and without S9 activation. All dose levels of test article, negative controls and positive controls will be plated in duplicate. Unless indicated otherwise by the Sponsor, the highest dose will be the highest workable concentration in the vehicle of choice but not to exceed 5 mg/plate. Solubility or workability permitting, the dose levels will be 5000, 1500, 500, 150, 50, 15, 5.0 and 1.5 µg per plate. In selecting dose levels for the confirmatory mutagenicity assay the following guidelines will be employed. Doses will be selected such that precipitate does not interfere with manual scoring. Whenever possible, the highest dose for the confirmatory mutagenicity assay will be selected to give some indication of toxicity without exceeding 5 mg/plate. For freely soluble, nontoxic test articles, the highest dose level will be 5 mg/plate. For precipitating, nontoxic test articles, the highest dose level may be selected in an

attempt to yield precipitate at only the top one or two dose levels. The Sponsor will be consulted regarding dose selection if (1) the maximum dose level is selected based on precipitation and this dose level is less than 5 mg/plate or (2) the maximum achievable test article dose level is less than 5 mg/plate and this dose level is nontoxic. The doses selected for the confirmatory mutagenicity assay will be documented in the raw data and report. If a retest of the initial toxicity-mutation assay is needed, a minimum of five dose levels of test article will be used in the retest.

### 7.3 Confirmatory Mutagenicity Assay

The test article will be tested at a minimum of five dose levels along with appropriate negative and positive controls with tester strains TA98, TA100, TA1535, TA1537 and WP2 *uvrA* with and without S9 activation. All dose levels of test article, negative controls and positive controls will be plated in triplicate.

### 7.4 Frequency and Route of Administration

The test system will be exposed to the test article via the preincubation modification of the Ames test described by Yahagi *et al.* (1977). This test system has been shown to detect a wide range of classes of chemical mutagens (McCann *et al.*, 1975; McCann and Ames, 1976).

If the Sponsor is aware of specific metabolic requirements (e.g., azo compounds), this information will be utilized in designing the assay. Verification of a clear positive response is not required. Equivocal results will be retested in consultation with the Sponsor using an appropriate modification of the experimental design (e.g., dose levels, activation system or treatment method). This guidance is based on the OECD Guideline 471 (1997).

### 7.5 Controls

No analyses will be performed on the positive control articles or the positive control dose formulations. The neat positive control articles and the vehicles used to prepare the test article and positive control formulations will be characterized by the Certificates of Analysis provided by the Supplier(s). Copies of the Certificates of Analysis will be kept on file at BioReliance.

#### 7.5.1 Positive Controls

The positive controls that will be plated concurrently with the assay are listed below. Results obtained from these articles will be used to assure responsiveness of the test system but not to provide a standard for comparison with the test article.

Strain	S9	Positive Control	Concentration (µg/plate)
<i>Salmonella</i> Strains	Rat	2-aminoanthracene	1.0-2.0
WP2 <i>uvrA</i>			10-20
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 <i>uvrA</i>		methyl methanesulfonate	1,000

#### 7.5.2 Negative Controls

Appropriate negative controls will be plated for each tester strain with and without S9 activation. The negative control will be the vehicle alone, unless there is no historical basis for use of the selected vehicle. In the latter case, both untreated and vehicle controls will be used.

#### 7.5.3 Sterility Controls

At a minimum, the most concentrated test article dilution and the Sham and S9 mixes will be checked for sterility.

### 7.6 Exogenous Metabolic Activation

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The S9 homogenate will be prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The S9 homogenate was or will be purchased from Molttox and stored frozen at -60°C or colder until used. Each batch of S9 homogenate was or will be assayed for its ability to metabolize at least two promutagens to forms mutagenic to *S. typhimurium* TA100.

Immediately prior to use, the S9 will be thawed and mixed with a cofactor pool to contain 10% S9 homogenate, 5 mM glucose-6-phosphate, 4 mM β-nicotinamide-adenine dinucleotide phosphate, 8 mM MgCl<sub>2</sub> and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4. This mixture is referred to as S9 mix. Sham mix will be 100 mM phosphate buffer at pH 7.4.

### 7.7 Preparation of Tester Strain

Each tester strain culture will be inoculated from the appropriate frozen stock, lyophilized pellet(s), or master plate. To ensure that cultures are harvested in late log phase, the length of incubation will be controlled and monitored. Each inoculated flask will be placed in a shaker/incubator programmed to begin shaking at 125 to 175 rpm and incubating at 37±2°C.

All cultures will be harvested by spectrophotometric monitoring of culture turbidity rather than by duration of incubation since overgrowth of cultures can cause loss of sensitivity to some mutagens. Cultures will be removed from incubation at a density of approximately  $10^9$  cells/mL.

#### 7.8 Test System Identification

Each plate will be labeled with a code system that identifies the test article, test phase, dose level, tester strain and activation type as described in BioReliance's Standard Operating Procedures.

#### 7.9 Test Article Preparation

Unless specified otherwise, test article dilutions will be prepared immediately prior to use. All test article dosing will be at room temperature under yellow light.

#### 7.10 Treatment of Test System

One-half milliliter (0.5 mL) of S9 mix or sham mix will be added to pre-heated 13 x 100 mm glass culture tubes. To these tubes will be added 100  $\mu$ L of tester strain and 50  $\mu$ L of vehicle, test article dilution or positive control. When necessary to achieve the target concentration or eliminate toxic vehicle effects, aliquots of other than 50  $\mu$ L of test article/vehicle/positive control will be plated. **Tubes receiving test article will be capped during the preincubation period.** After vortexing, the mixture will be allowed to incubate for **90 $\pm$ 2 minutes at 37 $\pm$ 2 $^{\circ}$ C with shaking.** Two milliliters of selective top agar will then be added to each tube and the mixture will be overlaid onto the surface of a minimal bottom agar plate. **After the overlay has solidified, the plates receiving test article will be inverted and placed in an appropriate number of 9-liter desiccators by dose level and incubated for 48 to 72 hours at 37 $\pm$ 2 $^{\circ}$ C.** Plates that are not counted immediately following the incubation period will be stored at 2-8 $^{\circ}$ C.

#### 7.11 Scoring

The condition of the bacterial background lawn will be evaluated for evidence of test article toxicity and precipitate. Evidence of toxicity will be scored relative to the negative control plate and recorded along with the revertant count for that plate. Toxicity will be evaluated as a decrease in the number of revertant colonies per plate and/or a thinning or disappearance of the bacterial background lawn. Precipitation will be evaluated after the incubation period by visual examination without magnification.

#### 7.12 Tester Strain Verification

On the day of use in the initial toxicity-mutation assay and the confirmatory mutagenicity assay, all tester strain cultures will be checked for the appropriate genetic markers cited in §6.0.

7.13 Automated Data Collection Systems

The primary computer or electronic systems used for the collection of data or analysis may include but are not limited to the following:

Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments), LIMS System (BioReliance), BRIQS (BioReliance), Excel 2007 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The following criteria must be met for the initial toxicity-mutation assay and the confirmatory mutagenicity assay to be considered valid. If one or more of these parameters are not acceptable, the affected condition(s) will be retested.

8.1 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 *uvrB* mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the *uvrA* mutation, all *E. coli* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the pKM101 plasmid R-factor, tester strain cultures of TA98 and TA100 must exhibit resistance to ampicillin.

8.2 Negative Controls Values

Based on historical control data, all tester strain cultures must exhibit characteristic numbers of spontaneous revertants per plate in the vehicle controls. The mean revertants per plate must be within the following ranges (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvrA*, 10 - 60. Untreated controls, when part of the design, must also be within the ranges cited above.

8.3 Tester Strain Titters

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

8.4 Positive Control Values

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

8.5 Toxicity

A minimum of three non-toxic dose levels will be required to evaluate assay data. A dose level is considered toxic if it causes a >50% reduction in the mean number

of revertants per plate relative to the mean vehicle control value (this reduction must be accompanied by an abrupt dose-dependent drop in the revertant count) or a reduction in the background lawn. In the event that less than three non-toxic dose levels are achieved, the affected portion of the assay will be repeated with an appropriate change in dose levels.

## 9.0 EVALUATION OF TEST RESULTS

For a test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

### 9.1 Strains TA1535 and TA1537

Data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value.

### 9.2 Strains TA98, TA100 and WP2 *uvrA*

Data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

## 10.0 REPORT

A report of the results of this study will accurately describe all methods used for generation and analysis of the data. The report will include, but not be limited to information about the following:

- Test article
- Vehicle
- Strains
- Test conditions
- Results
- Discussion of results
- Conclusion
- Appendices: Historical Control Data (vehicle and positive controls with ranges, means and standard deviations), copy of protocol and any amendment, contributing reports (if applicable), and, if provided by the Sponsor, copies of the analyses that

characterized the test article, its stability and the stability and strength of the dosing preparations.

- Statement of Compliance
- Quality Assurance Statement
- CTD Tables (unless otherwise requested)

The report will be issued as a QA-audited draft. After receipt of the Sponsor's comments a final report will be issued. A GLP Compliance Statement signed by the Study Director will also be included in the final report and will note any exceptions if the characterization of the test article and/or the characterization of the dose formulations are not performed or provided. Six months after issuance of the draft report, if no communication regarding the study is received from the Sponsor or designated representative, the draft report may be issued as a final report. If all supporting documents have not been provided, the report will be written based on those that are provided.

#### 11.0 RECORDS AND ARCHIVES

All raw data, the protocol, pertinent study email correspondence, slides and/or specimens (as applicable), and all reports for procedures performed at BioReliance will be maintained in the archives at BioReliance, Rockville, MD for at least five years. At that time, the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials will first be copied and the copy will be retained by the BioReliance archives in accordance with the applicable SOPs. The raw data, reports, and other documents generated at locations other than BioReliance will be archived by the test site.

#### 12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, adopted July 21, 1997.

This study will be performed in compliance with the following Good Laboratory Practices (GLP) regulations.

- US FDA Good Laboratory Practices 21 CFR Part 58
- OECD Principles of Good Laboratory Practice (C(97)186/Final)

At a minimum, all work performed at US test site(s) will comply with the US GLP regulations stated above. Non-US sites must follow the GLP regulations governing their site. The regulations that were followed will be indicated on the compliance statement in the final contributing report.

### 13.0 REFERENCES

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.

Green, M.H.L., and Muriel, W.J. (1976). Mutagen testing using *trp*<sup>+</sup> reversion in *Escherichia coli*. *Mutation Research* 38:3-32.

McCann, J. and Ames, B.N. (1976). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion. *Proc. Natl. Acad. Sci. USA* 73:950-954.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc. Natl. Acad. Sci. USA* 72:5135-5139.

OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, adopted July 21, 1997.

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.

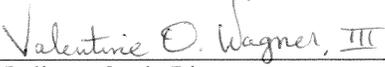
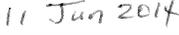
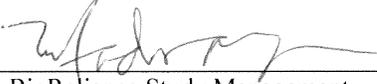
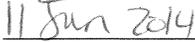
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14.0 APPROVALS

14.1 Sponsor Approval

Caro J. Smith                      June 11, 2014  
Sponsor Representative                      Date

14.2 Study Director and Test Facility Management Approvals

 _____ BioReliance Study Director	 _____ Date
 _____ BioReliance Study Management	 _____ Date

**APPENDIX III: Certificate of Analysis**

# ALBEMARLE

ALBEMARLE CORPORATION  
451 FLORIDA STREET Phone: 225-388-8011  
BATON ROUGE, 70801 Fax: 225-388-7686

BIORELIANCE CORPORATION  
ATTN: ALBERT BREW-HAGAN  
9630 MEDICAL CENTER DR. TOXICOLOGY  
ROCKVILLE MD 20850

Contact person fax:

## Certificate of Analysis

Certificate Date  
08/15/2013  
Purchase order item/date  
REQUESTED BY JOE MILLER / 08/13/2013  
Delivery item/goods issue date  
81110601 000001 / 08/23/2013  
Order item  
6332221 000001  
Customer number  
AG-400134 WE-400134

Page 1 of 1

Material: Our / Your reference  
8057 N-PROPYL BROMIDE/BULK /

THE EXPIRATION DATE FOR THIS SAMPLE IS FEBRUARY 12, 2015

Inspection lot 100000139331 from 08/14/2013 Quantity 0.300 KG

Characteristic	Unit	Value	Lower Limit	Upper Limit	Method
Color APHA	APHA	3	-	50	
n-Propyl Bromide	Wt%	100.0	99.0	-	
Acidity (as HBr)	ppm	4	-	10	
Water	ppm	27	-	100	

This certificate is computer generated and produced without a signature, from results validated by the Quality Control and Assurance Process.

The above data are the results of our quality inspection and testing. They do not relieve the customer from inspecting and testing the product on its receipt and do not imply a guarantee of its suitability for a particular use.

**APPENDIX IV: Dosing Formulation Analysis and Stability**

**Bacterial Reverse Mutation Assay**

**Determination of 1-bromopropane in Ethanol Dosing Formulations and 1-bromopropane in 100 mM Phosphate Buffer (pH 7.4) Treatment Samples**

AUDITED DRAFT ANALYTICAL REPORT

Test Article

1-bromopropane

Author

Philip Atkins, MChem

Draft Analytical Report Date

20-Aug-2014

Analytical Laboratory

BioReliance Corporation  
9610 Medical Center Drive  
Rockville, MD 20850

BioReliance Study Number

AD58PU.503001.BTL

Sponsor

Albemarle Corporation  
451 Florida Street  
Baton Rouge, LA 70801

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### Compliance Statement

This portion of the study was conducted in compliance with the provisions of the most recent version of the US FDA Good Laboratory Practices 21 CFR Part 58 and OECD Principles of Good Laboratory Practice (C(97) 186/Final).

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Philip Atkins, MChem.  
Contributing Scientist  
BioReliance

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Date

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### **Analytical Conditions**

The analysis of the test article formulation samples, 1-bromopropane in ethanol, and the treatment samples, 1-bromopropane in 100 mM phosphate buffer (pH 7.4), for study AD58PU.503001.BTL was performed by Gas chromatography (GC) using a method validated under BioReliance Study Number AD58PU.GGTACHEM.BTL. The formulations were also analyzed in accordance with BioReliance SOP OPAC0385 "Dose Formulation Analysis" and OPAC0388 "Dose Formulation Stability Determination". The analytical conditions used in this study are summarized in [Table 1](#) and [Table 2](#). The matrix standards were prepared per [Table 3](#) and [Table 4](#).

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Table 1: Analytical Conditions in Ethanol

Instrument:	Agilent 6890N Gas Chromatograph	
Detector:	Flame Ionization (FID)	
Software:	Agilent C/S ChemStore Rev. B.03.03 and ChemStation Revision B.02.01-SR2 software with data security add-on	
Diluent:	Ethanol	
Vehicle:	Ethanol	
(SS) Stock Solution:	200 µg/mL Test Article in Diluent	
(IS) Internal Standard:	p-Cymene: Ethanol (25:75, v/v)	
TA Correction Factor :	0.73855	
TA Density:	1.354 g/mL <sup>1</sup>	
Inlet Temperature:	200°C	
Inlet Pressure:	3 psi	
Split Ratio:	1:1	
Split Flow:	3.9 mL/min	
Total Inlet Flow:	10.5 mL/min	
Inlet Gas:	Helium	
Column:	Restek RTX-624, 30m x 530 µm, 3µm film thickness	
Column Flow Mode:	Constant Flow	
Flow rate:	3.9 mL/min	
Detector Temperature:	275° C	
Hydrogen Flow:	35 mL/min	
Air Flow:	300 mL/min	
Detector Flow Mode:	Constant Flow	
Makeup Flow:	10 mL/min	
Makeup Gas:	Helium	
Syringe Needle Washes (A & B):	Isopropanol (IPA)	
Injection Volume:	1 µL	
Pre-Injection Solvent A Washes:	2	
Post-Injection Solvent A Washes:	2	
Pre-Injection Solvent B Washes:	2	
Post-Injection Solvent B Washes:	2	
Injections/Sample:	2	
Run Time:	21.5 minutes	
Test Article Retention Time:	~6.97 minutes	
Calibration Curve:	y = Ax + B (not weighted)	
Oven Temperature Profile:	Gradient (see below)	
Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
0	45	4
10	200	2

<sup>1</sup>TA measured by volume and corrected for density.

Table 2: Analytical Conditions for Phosphate Buffer

Instrument:	Agilent 6890N Gas Chromatograph	
Detector:	Flame Ionization (FID)	
Software:	Agilent C/S ChemStore Rev. B.03.03 and ChemStation Revision B.02.01-SR2 software with data security add-on	
Diluent:	Ethanol	
Vehicle:	100 mM Phosphate Buffer, pH 7.4	
(SS) Stock Solution:	150 µg/mL Test Article in Diluent	
(IS) Internal Standard:	p-Cymene: Ethanol (25:75, v/v)	
TA Correction Factor :	0.73855	
TA Density:	1.354 g/mL <sup>1</sup>	
Inlet Temperature:	185°C	
Inlet Pressure:	3.5 psi	
Split Ratio:	1:1	
Split Flow:	4.6 mL/min	
Total Inlet Flow:	11.9 mL/min	
Inlet Gas:	Helium	
Column:	Restek RTX-624 30m x 530 µm, 3µm film thickness	
Column Flow Mode:	Constant Flow	
Flow rate:	4.6 mL/min	
Detector Temperature:	275° C	
Hydrogen Flow:	35 mL/min	
Air Flow:	300 mL/min	
Detector Flow Mode:	Constant Flow	
Makeup Flow:	10 mL/min	
Makeup Gas:	Helium	
Syringe Needle Washes (A & B):	Isopropanol (IPA)	
Injection Volume:	1 µL	
Pre-Injection Solvent A Washes:	2	
Post-Injection Solvent A Washes:	2	
Pre-Injection Solvent B Washes:	2	
Post-Injection Solvent B Washes:	2	
Injections/Sample:	2	
Run Time:	27.5 minutes	
Test Article Retention Time:	~6.36 minutes	
Calibration Curve:	y = Ax + B (not weighted)	
Oven Temperature Profile:	Gradient (see below)	
Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
0	45	4
5	140	2
40	200	1

<sup>1</sup>TA measured by volume and corrected for density.

Table 3: Preparation of the Matrix Standard Solutions in Ethanol

Standard ID	IS (mL)	SS (mL)	Final Volume with Diluent (mL)	Final TA Concentration (µg/mL)
M-0	0	0	10	0
M-1	1	0	10	0
M-1	1	1	10	20
M-2	1	2	10	40
M-3	1	3	10	60
M-4	1	4	10	80
M-5	1	5	10	100
M-6	1	6	10	120

Table 4: Preparation of the Matrix Standard Solutions in Phosphate Buffer

Standard ID	IS (mL)	SS (mL)	Vehicle (mL)	Final Volume with Diluent (mL)	Final TA Concentration (µg/mL)
M-0	0	0	1	10	0
M-1	1	0	1	10	0
M-1	1	1	1	10	15
M-2	1	2	1	10	30
M-3	1	3	1	10	45
M-4	1	4	1	10	60
M-5	1	5	1	10	75
M-6	1	5.5	1	10	82.5

### Dosing Formulation and Treatment Samples Analysis

Dosing formulations of 1-bromopropane in ethanol and treatment samples of 1-bromopropane in 100 mM Phosphate Buffer, pH 7.4 were collected and analyzed by GC on the day of preparation to assess accuracy of the preparation (per Table 5). A sample of vehicle dosing solution from each experiment was also analyzed to verify that it did not contain test article.

The dosing formulations and treatment samples were diluted to bring the test article concentration to a suitable level within the calibration range and with the same final composition as the matrix standards. The concentration of 1-bromopropane was calculated by reference to the matrix standard solutions prepared (per Table 3 and Table 4) and analyzed concurrently with the dosing formulations and treatment samples. All matrix standard curves met the acceptance criteria (Table 6, Table 8, Table 10, Table 12, Table 14 and Table 16).

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All dosing formulations analyzed met the acceptance criteria of 85.0-115.0% of target concentration and  $\leq 5.00\%$  RSD. No test article was detected in the vehicle control (VC) samples, except in the vehicle control sample for the Experiment B3 in which a small peak of the test article was detected ([Table 7](#), [Table 11](#) and [Table 15](#)).

The treatment samples in 100 mM Phosphate Buffer, pH 7.4 for Experiments B1, B2 and B3 were analyzed and results are shown in [Table 9](#), [Table 13](#) and [Table 17](#). No acceptance criteria were applied for the analysis of the treatment samples.

Table 5: Summary of the Dosing Formulations

Experiment No./Phase	Date of Preparation	Date of Analysis (Start/End)	Concentration (mg/mL)	Homogeneity Testing (Y) Yes or (N) No
B1	18-Jun-2014	18-Jun-2014/ 19-Jun-2014	0	N
			0.060	N
			200	N
B1 (Start of Pre-Incubation)	18-Jun-2014	18-Jun-2014/ 21-Jun-2014	0	N
			0.95	N
			9.5	N
B1 (End of Pre-Incubation)	18-Jun-2014	18-Jun-2014/ 21-Jun-2014	0	N
			0.95	N
			9.5	N
B2	27-Jun-2014	27-Jun-2014/ 28-Jun-2014	0	N
			2.0	N
			200	N
B2 (Start of Pre-Incubation)	27-Jun-2014	27-Jun-2014/ 29-Jun-2014	0	N
			0.95	N
			9.5	N
B2 (End of Pre-Incubation)	27-Jun-2014	27-Jun-2014/ 29-Jun-2014	0	N
			0.95	N
			9.5	N
B3	24-Jul-2014	24-Jul-2014/ 26-Jul-2014	0	N
			2.0	N
			200	N
B3 (Start of Pre-Incubation)	24-Jul-2014	24-Jul-2014/ 01-Aug-2014	0	N
			0.95	N
			9.5	N
B3 (End of Pre-Incubation)	24-Jul-2014	24-Jul-2014/ 01-Aug-2014	0	N
			0.95	N
			9.5	N

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Table 6: Matrix Standards for the B1 Dosing Formulation and Stability Analyses in Ethanol

Item	Value	Acceptance Criterion
Slope	0.0009792	NA
Intercept	0.0002397	NA
Correlation Coefficient	0.9998	>0.99
Recovery % (Range)	98.4-101.4	90-110

Table 7: B1 Dosing Formulation Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA/IS Peak Area Ratio	Mean Peak Area Ratio	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
VC*	0	0	ND						
VC	0	0	ND	ND	NA	NA	2	NA	NA
0.060 A	0.060	24.00	0.02410						
0.060 A	0.060	24.00	0.02430						
0.060 B	0.060	24.00	0.02506						
0.060 B	0.060	24.00	0.02528	0.02469	24.97	104.0	2.500	0.0624	2.32
200 A	200	100.0	0.09399						
200 A	200	100.0	0.09242						
200 B	200	100.0	0.09653						
200 B	200	100.0	0.09701	0.09499	96.76	96.8	2000	194	2.28

\*See Deviation [Event #188520](#).

Table 8: Matrix Standards for the B1 Treatment Samples in Phosphate Buffer

Item	Value	Acceptance Criterion
Slope	0.001156	NA
Intercept	-0.001312	NA
Correlation Coefficient	0.9990	>0.99
Recovery % (Range)	98.5-102.0	90-110

See Deviation [Event #188521](#)

Table 9: B1 Treatment Samples Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA/IS Peak Area Ratio	Mean Peak Area Ratio	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
VC (Start, T=0)	0	0	ND						
VC (Start, T=0)	0	0	ND	ND	NA	NA	10	NA	NA
VC (End, T=90 min)	0	0	ND						
VC (End, T=90 min)	0	0	ND	ND	NA	NA	10	NA	NA
0.95 (Start, T=0) A	0.95	19	0.001015						
0.95 (Start, T=0) A	0.95	19	0.000757						
0.95 (Start, T=0) B	0.95	19	0.001211						
0.95 (Start, T=0) B	0.95	19	0.001151	0.001034	2.029	10.7	50	0.101	19.51
9.5 (Start, T=0) A	9.5	47.50	0.003080						
9.5 (Start, T=0) A	9.5	47.50	0.003423						
9.5 (Start, T=0) B	9.5	47.50	0.003437						
9.5 (Start, T=0) B	9.5	47.50	0.003380	0.003330	4.016	8.5	200	0.803	5.06
0.95 (End, T=90 min) A	0.102	2.030	0.001077						
0.95 (End, T=90 min) A	0.102	2.030	0.001065						
0.95 (End, T=90 min) B	0.102	2.030	0.001504						
0.95 (End, T=90 min) B	0.102	2.030	0.001605	0.001313	2.271	111.9	50.25	0.114	21.49
9.5 (End, T=90 min) A	0.803	4.016	0.004183						
9.5 (End, T=90 min) A	0.803	4.016	0.004114						
9.5 (End, T=90 min) B	0.803	4.016	0.004181						
9.5 (End, T=90 min) B	0.803	4.016	0.004076	0.004139	4.715	117.4	200.0	0.943	1.27

<sup>1</sup>Concentrations for end of incubation samples (T=90 mins) were determined at start of incubation (T=0)

Table 10: Matrix Standards for the B2 Dosing Formulation in Ethanol

Item	Value	Acceptance Criterion
Slope	0.001150	NA
Intercept	0.0003227	NA
Correlation Coefficient	0.9965	>0.99
Recovery % (Range)	96.6-106.1	90-110

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Table 11: B2 Dosing Formulation Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA/IS Peak Area Ratio	Mean Peak Area Ratio	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
VC	0	0	ND						
VC	0	0	ND	ND	NA	NA	2	NA	NA
2.0 A	2.0	80	0.09257						
2.0 A	2.0	80	0.09427						
2.0 B	2.0	80	0.09529						
2.0 B	2.0	80	0.09485	0.09425	81.68	102.1	25	2.04	1.26
200 A	200	100	0.1146						
200 A	200	100	0.1193						
200 B	200	100	0.1222						
200 B	200	100	0.1194	0.1189	103.1	103.1	2000	206	2.65

Table 12: Matrix Standards for the B2 Treatment Samples in Phosphate Buffer

Item	Value	Acceptance Criterion
Slope	0.001156	NA
Intercept	-0.001312	NA
Correlation Coefficient	0.9990	>0.99
Recovery % (Range)	98.5-102.0	90-110

Table 13: B2 Treatment Samples Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA/IS Peak Area Ratio	Mean Peak Area Ratio	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
VC (Start, T=0)	0	0	ND						
VC (Start, T=0)	0	0	ND	ND	NA	NA	10	NA	NA
VC (End, T=90 min)	0	0	ND						
VC (End, T=90 min)	0	0	ND	ND	NA	NA	10	NA	NA
0.95 (Start, T=0) A	0.95	76	0.02983						
0.95 (Start, T=0) A	0.95	76	0.03361						
0.95 (Start, T=0) B	0.95	76	0.03606						
0.95 (Start, T=0) B	0.95	76	0.03335	0.03321	28.27	37.2	12.50	0.353	7.72
9.5 (Start, T=0) A	9.5	63.33	0.007796						
9.5 (Start, T=0) A	9.5	63.33	0.007442						
9.5 (Start, T=0) B	9.5	63.33	0.007178						
9.5 (Start, T=0) B	9.5	63.33	0.007383	0.007450	5.847	9.2	150.0	0.877	3.45
0.95 (End, T=90 min) A	0.353	28.27	0.002891						
0.95 (End, T=90 min) A	0.353	28.27	0.002845						
0.95 (End, T=90 min) B	0.353	28.27	0.002989						
0.95 (End, T=90 min) B	0.353	28.27	0.003143	0.002967	1.945	6.9	12.49	0.0243	4.44
9.5 (End, T=90 min) A	0.877	5.847	0.002340						
9.5 (End, T=90 min) A	0.877	5.847	0.001998						
9.5 (End, T=90 min) B	0.877	5.847	0.002219						
9.5 (End, T=90 min) B	0.877	5.847	0.002508	0.002266	1.335	22.8	150.0	0.200	9.47

<sup>1</sup>Concentrations for end of incubation samples (T=90 mins) were determined at start of incubation (T=0)

Table 14: Matrix Standards for the B3 Dosing Formulation in Ethanol

Item	Value	Acceptance Criterion
Slope	0.001016	NA
Intercept	-0.002052	NA
Correlation Coefficient	0.9931	>0.99
Recovery % (Range)	95.2-107.8	90-110

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Table 15: B3 Dosing Formulation Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA/IS Peak Area Ratio	Mean Peak Area Ratio	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
VC	0	0	0.01504						
VC	0	0	0.01473	0.01489	16.68	NA	2	0.0334	NA
2.0 A	2.0	80	0.07185						
2.0 A	2.0	80	0.07515						
2.0 B	2.0	80	0.08102						
2.0 B	2.0	80	0.07893	0.07674	77.55	96.9	25	1.94	5.30
200 A	200	100	0.09494						
200 A	200	100	0.09590						
200 B	200	100	0.09640						
200 B	200	100	0.1025	0.09744	97.93	97.9	2000	196	3.52

Table 16: Matrix Standards for the B3 Treatment Samples in Phosphate Buffer

Item	Value	Acceptance Criterion
Slope	0.001004	NA
Intercept	0.001860	NA
Correlation Coefficient	0.9982	>0.99
Recovery % (Range)	92.8-102.4	90-110

Table 17: B3 Treatment Samples Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA/IS Peak Area Ratio	Mean Peak Area Ratio	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
VC (Start, T=0)	0	0	ND						
VC (Start, T=0)	0	0	ND	ND	NA	NA	10	NA	NA
VC (End, T=90 min)	0	0	ND						
VC (End, T=90 min)	0	0	ND	ND	NA	NA	10	NA	NA
0.95 (Start, T=0) A	0.95	76	0.007635						
0.95 (Start, T=0) A	0.95	76	0.007650						
0.95 (Start, T=0) B	0.95	76	0.007605						
0.95 (Start, T=0) B	0.95	76	0.007368	0.007565	5.682	7.5	12.50	0.0710	1.75
9.5 (Start, T=0) A	9.5	63.333	0.004557						
9.5 (Start, T=0) A	9.5	63.333	0.004415						
9.5 (Start, T=0) B	9.5	63.333	0.004496						
9.5 (Start, T=0) B	9.5	63.333	0.004390	0.004465	2.595	4.1	150.0	0.389	1.71
0.95 (End, T=90 min) A	0.0710	5.682	0.005515						
0.95 (End, T=90 min) A	0.0710	5.682	0.005470						
0.95 (End, T=90 min) B	0.0710	5.682	0.005129						
0.95 (End, T=90 min) B	0.0710	5.682	0.005260	0.005344	3.470	61.1	12.50	0.0434	3.39
9.5 (End, T=90 min) A	0.389	2.595	0.003438						
9.5 (End, T=90 min) A	0.389	2.595	0.003730						
9.5 (End, T=90 min) B	0.389	2.595	0.003858						
9.5 (End, T=90 min) B	0.389	2.595	0.003693	0.003680	1.813	69.9	149.9	0.272	4.78

<sup>1</sup>Concentrations for end of incubation samples (T=90 mins) were determined at start of incubation (T=0)

### Stability of 1-bromopropane in Ethanol Dosing Formulations

Stability of the dosing formulations in ethanol was determined by storing the B1 dosing formulations at room temperature for T=3.25 hours and reanalyzing as described above. The acceptance criterion of 90-110% of the concentration determined at T=0 was met (Table 18).

1-bromopropane in Ethanol, at concentrations of 0.0624 and 194 mg/mL, was stable at room temperature for at least 3.25 hours.

Table 18: Stability T=3.25 Hours Analysis

Formulation ID	Conc. of Form. <sup>1</sup> (mg/mL)	Conc. of Sample <sup>1</sup> (µg/mL)	TA Peak Area	Mean Peak Area	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	RSD (%)
0.060 A	0.0624	24.97	0.02327						
0.060 A	0.0624	24.97	0.02450						
0.060 B	0.0624	24.97	0.02279						
0.060 B	0.0624	24.97	0.02404	0.02365	23.91	95.8	2.499	0.0598	3.24
200 A	194	96.76	0.10000						
200 A	194	96.76	0.09735						
200 B	194	96.76	0.09807						
200 B	194	96.76	0.09703	0.09811	99.95	103.3	2005	200	1.36

<sup>1</sup>Concentration determined at T=0 (Table 7)

### Stability of 1-bromopropane in 100 mM Phosphate Buffer (pH 7.4)

Stability of the test article in 100 mM phosphate buffer (PH 7.4) was determined as part of BioReliance Study AD58PU.GCGTCHEM.BTL

The stability of 1-bromopropane in 100 mM phosphate buffer (PH 7.4), at concentrations of 7.408, 2.778, 0.7408, 0.2778 and 0.07408 mg/mL, when stored at 37±2°C for either T=30, T=60, T=90 or T=120 minutes were not established, except for 0.07408 mg/mL at T=30 minutes storage which met the acceptance criterion of 90-110% of target (initial concentration at T=0).

### Conclusion

For the analysis of the dosing formulations, the submitted formulations were found to be accurately prepared. The vehicle control samples were free of test article, except for the Experiment B3 vehicle control sample in which a small peak of the test article was detected. The treatment samples in 100 mM Phosphate Buffer, pH 7.4 for Experiments B1, B2 and B3 were collected and analyzed. Additionally, 1-bromopropane in Ethanol, at concentrations of 0.0624 and 194 mg/mL, was stable at room temperature for at least 3.25 hours

### Deviations

Two deviations occurred during the conduct of this portion of the study.

Event# 188520: During dilution of Experiment B1 vehicle control samples for analysis in ethanol, no internal standard solution was added to the samples. This deviated from BioReliance SOP OPAC0385.

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Event# 188521: The M-I matrix standard was not analyzed during the analysis of matrix standards for Experiment B1 treatment samples in phosphate buffer. This deviated from BioReliance SOP OPAC0385.

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## Abbreviations and Calculations

Calc. = Calculated  
Conc. = Concentration  
Form = Formulation  
HPLC = High Performance Liquid Chromatography  
M = Matrix  
NA = Not Applicable  
ND = Not Detected  
RSD = Relative Standard Deviation  
T = Time  
TA = Test Article  
UV = Ultra-Violet  
VC = Vehicle Control

The following formulas were used for the calculations:

1. Mean Concentration ( $\mu\text{g}/\text{mL}$ ) = (Mean Peak Area - Intercept) / Slope  
Intercept and slope calculated using linear regression analysis
2. Final Mean Conc. ( $\text{mg}/\text{mL}$ ) = (Mean Conc. ( $\mu\text{g}/\text{mL}$ ) x Dilution Factor) / 1000
3. % of Target, % Recovery =  $\frac{\text{Mean Concentration}}{\text{Concentration of Sample}} \times 100$
4. % RSD =  $\frac{\text{Standard Deviation of the TA Peak Area}}{\text{Mean Peak Area}} \times 100$

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**APPENDIX V: Common Technical Document Tables**

2.6.7.8 Genotoxicity: In Vitro

Study No: AD58PU.503001.BTL

Test Article: 1-bromopropane

**Test for Induction of:** Reverse mutation in bacterial cells

**Species/Strain:** *S. typhimurium*, *E. coli*

**Metabolizing System:** Aroclor-induced rat liver S9

**Vehicle for Test Article:** EtOH

**Treatment:** Preincubation (90±2 minutes at 37±2°C)

**Cytotoxic Effects:** Toxicity was observed at 5000 µg per plate with all *Salmonella* tester strains in the initial and confirmatory assays (#1 and 2) and beginning at 3000 or at 5000 µg per plate with all tester strains in the retest of the confirmatory assay (#3).

**Genotoxic Effects:** None

**No. of Independent Assays:** 3

**No. of Replicate Cultures:** 2 (#1) and 3 (#2 and 3)

**No. of Cells Analyzed/Culture:** 1.3 to 5.7 x 10<sup>8</sup> cells/plate

**Vehicle for Positive Controls:** DMSO, except sterile water for sodium azide

**Dates of Treatment:** 18 June 2014 (#1), 27 June 2014 (#2) and 24 July 2014 (#3)

**Study No.:** AD58PU.503001.BTL

**Location in CTD:**

**GLP Compliance:** Yes

<u>Metabolic Activation</u>	<u>Test Article</u>	<u>Dose Level (µg/plate)</u>	<u>Initial Toxicity-Mutation Assay (#1) Revertant Colony Counts (Mean ±SD)</u>				
			<u>TA98</u>	<u>TA100</u>	<u>TA1535</u>	<u>TA1537</u>	<u>WP2uvrA</u>
Without Activation	Ethanol 1-bromopropane	25 µL/plate	14 ± 1	88 ± 11	12 ± 1	9 ± 3	24 ± 1
		1.5	15 ± 1	77 ± 20	12 ± 4	6 ± 0	17 ± 1
		5.0	22 ± 8	84 ± 13	9 ± 3	7 ± 1	20 ± 1
		15	15 ± 3	77 ± 4	7 ± 2	4 ± 4	22 ± 1
		50	16 ± 2	96 ± 1	11 ± 4	8 ± 1	24 ± 1
		150	22 ± 4	74 ± 6	13 ± 3	6 ± 1	19 ± 8
		500	12 ± 3	106 ± 1	9 ± 1	6 ± 1	26 ± 5
		1500	17 ± 1	71 ± 0	14 ± 1	5 ± 2	20 ± 4
		5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	23 ± 6
		2NF	1.0	761 ± 65			
	SA	1.0		591 ± 32	602 ± 26		
	9AAD	75				259 ± 81	
	MMS	1000					563 ± 33

Key to Positive Controls

SA	sodium azide
9AAD	9-Aminoacridine
2NF	2-nitrofluorene
MMS	methyl methanesulfonate

## 2.6.7.8 Genotoxicity: In Vitro

Study No: AD58PU.503001.BTL

Test Article: 1-bromopropane

Metabolic Activation	Test Article	Dose Level (µg/plate)	Initial Toxicity-Mutation Assay (#1) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2 <sub>uvrA</sub>
With Activation	Ethanol 1-bromopropane	25 µL/plate	29 ± 7	104 ± 0	11 ± 11	12 ± 4	27 ± 3
		1.5	23 ± 2	86 ± 16	11 ± 6	10 ± 1	26 ± 6
		5.0	24 ± 2	93 ± 6	9 ± 0	8 ± 1	23 ± 1
		15	26 ± 11	103 ± 17	15 ± 1	11 ± 1	30 ± 1
		50	20 ± 6	89 ± 1	12 ± 7	15 ± 1	28 ± 1
		150	30 ± 0	100 ± 17	13 ± 0	9 ± 1	29 ± 2
		500	29 ± 5	103 ± 8	6 ± 1	9 ± 3	34 ± 13
		1500	27 ± 6	95 ± 16	13 ± 8	12 ± 1	32 ± 2
		5000	14 ± 6	63 ± 10	10 ± 2	8 ± 4	36 ± 3
		2AA	1.0	435 ± 5		52 ± 1	
	2AA	2.0		504 ± 60		47 ± 1	
	2AA	15					310 ± 2
	<b>Key to Positive Controls</b>						
2AA	2-aminoanthracene						

## 2.6.7.8 Genotoxicity: In Vitro

Study No: AD58PU.503001.BTL

Test Article: 1-bromopropane

Metabolic Activation	Test Article	Dose Level (µg/plate)	Confirmatory Mutagenicity Assay (#2) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Ethanol 1-bromopropane	25 µL/plate	24 ± 3	97 ± 12	13 ± 2	8 ± 3	25 ± 5
		50	21 ± 3	90 ± 8	11 ± 3	11 ± 3	27 ± 3
		150	19 ± 3	91 ± 8	10 ± 2	11 ± 3	32 ± 10
		500	23 ± 7	80 ± 16	17 ± 1	8 ± 4	30 ± 13
		1500	27 ± 1	72 ± 22	13 ± 1	6 ± 6	33 ± 3
		2000	24 ± 10	65 ± 20	8 ± 4	8 ± 1	32 ± 6
		3000	19 ± 7	67 ± 17	10 ± 4	7 ± 3	21 ± 5
		5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	21 ± 5
	2NF	1.0	624 ± 28				
	SA	1.0		1120 ± 55	836 ± 37		
	9AAD	75				312 ± 184	
	MMS	1000					561 ± 8
	With Activation	Ethanol 1-bromopropane	25 µL/plate	14 ± 4	97 ± 9	13 ± 5	13 ± 3
50			13 ± 5	106 ± 11	12 ± 5	5 ± 5	34 ± 8
150			14 ± 3	107 ± 10	12 ± 5	7 ± 4	32 ± 8
500			10 ± 1	108 ± 16	13 ± 3	6 ± 1	36 ± 1
1500			14 ± 3	85 ± 18	21 ± 8	8 ± 2	34 ± 6
2000			14 ± 1	81 ± 26	21 ± 3	7 ± 6	39 ± 11
3000			14 ± 2	79 ± 22	15 ± 3	6 ± 1	40 ± 12
5000			9 ± 3	59 ± 5 <sup>a</sup>	17 ± 1	12 ± 3 <sup>a</sup>	31 ± 2
2AA		1.0	519 ± 49		63 ± 3		
2AA		2.0		328 ± 35		40 ± 7	
2AA	15					334 ± 83	

## Key to Positive Controls

SA	sodium azide
2AA	2-aminoanthracene
9AAD	9-Aminoacridine
2NF	2-nitrofluorene
MMS	methyl methanesulfonate

<sup>a</sup> One replicate plate was lost due to the absence of bacterial cells (TA100) or due to contamination (TA1537). Therefore, standard deviation was calculated where n=2.

## 2.6.7.8 Genotoxicity: In Vitro

Study No: AD58PU.503001.BTL

Test Article: 1-bromopropane

Metabolic Activation	Test Article	Dose Level (µg/plate)	Retest of the Confirmatory Mutagenicity Assay (#3) Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Ethanol 1-bromopropane	25 µL/plate	22 ± 5	106 ± 12	15 ± 4	6 ± 1	32 ± 2
		50	39 ± 5	75 ± 9	15 ± 1	4 ± 1	26 ± 5 <sup>a</sup>
		150	37 ± 7	83 ± 12	18 ± 3	3 ± 1	27 ± 6
		500	36 ± 1	90 ± 12	18 ± 6	4 ± 1	32 ± 6
		1500	35 ± 6	74 ± 5	16 ± 4	5 ± 2	24 ± 2
		2000	30 ± 16	75 ± 3	13 ± 4	3 ± 1	29 ± 7
		3000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	33 ± 13
		5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	2NF	1.0	768 ± 62				
	SA	1.0		1057 ± 60	463 ± 5		
	9AAD	75				115 ± 2	
	MMS	1000					614 ± 58
	With Activation	Ethanol 1-bromopropane	25 µL/plate	32 ± 6	100 ± 32	31 ± 7	5 ± 1
50			25 ± 5	106 ± 27 <sup>a</sup>	26 ± 3	4 ± 1	32 ± 9
150			24 ± 3	88 ± 13	32 ± 3	4 ± 1	33 ± 5
500			25 ± 4	88 ± 11	24 ± 2	4 ± 2	25 ± 2
1500			25 ± 3	78 ± 14	20 ± 6	5 ± 2	28 ± 4
2000			23 ± 8	68 ± 6	20 ± 3	7 ± 2	31 ± 10
3000			0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5000			0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2AA		1.0	490 ± 7		105 ± 12		
2AA		2.0		554 ± 53		87 ± 16	
2AA	15					402 ± 15	

## Key to Positive Controls

SA	sodium azide
2AA	2-aminoanthracene
9AAD	9-Aminoacridine
2NF	2-nitrofluorene
MMS	methyl methanesulfonate

<sup>a</sup> One replicate plate was lost due to contamination. Therefore, standard deviation was calculated where n=2.