

FINAL REPORT

Study Title

Bacterial Reverse Mutation Assay of 1-Chlorododecane

Test Article

1-Chlorododecane

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Study Completion Date

21 May 2008

Testing Facility

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BioReliance Study Number

AC01UM.503.BTL

Sponsor

Lonza, Inc.  
90 Boroline Road  
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**STATEMENT OF COMPLIANCE**

Study No. AC01UM.503.BTL was conducted in compliance with the U.S. FDA GLP Regulations as published in 21 CFR 58, the U.S. EPA GLP Standard 40 CFR 792 and the OECD Principles of Good Laboratory Practice in all material aspects with the following exception:

Analyses to determine the uniformity or concentration of the test article mixtures and their stability were not performed by the testing facility or the Sponsor.

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

21 May 2008  
Date

Ramadevi Gudi  
BioReliance Study Management

21 May 2008  
Date

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Sponsor/Submitter

5/22/08  
Date

# Quality Assurance Statement

**Study Title:** Bacterial Reverse Mutation Assay of 1-Chlorododecane

**Study Number:** AC01UM.503.BTL

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

Quality Assurance performed the inspections listed below for this study. Verification of the study protocol was also performed and documented by QA. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standard 40 CFR 792 and the OECD Principles of Good Laboratory Practice, and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

**Inspect On:** 29-Aug-07 - 29-Aug-07 To Study Dir 29-Aug-07 To Mgmt 30-Aug-07  
**Phase:** Scoring the plates

**Inspect On:** 17-Oct-07 - 17-Oct-07 To Study Dir 17-Oct-07 To Mgmt 17-Oct-07  
**Phase:** Draft Report and Data Audit

**Inspect On:** 16-May-08 - 16-May-08 To Study Dir 16-May-08 To Mgmt 21-May-08  
**Phase:** Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.



Allison Schaefer, BS  
QUALITY ASSURANCE

21MAY108

DATE

**Bacterial Reverse Mutation Assay of 1-Chlorododecane**

STUDY INFORMATION

Sponsor: **Lonza, Inc.**  
**90 Boroline Road**  
**Allendale, NJ 07401**

Authorized Representative: **John P. Van Miller, Ph.D., DABT**  
**Toxicology Regulatory Services, Inc.**

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

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

Test Article Batch No.: **N6227945**

Test Article CAS No.: **112-52-7**

BioReliance Study No.: **AC01UM.503.BTL**

Test Article Description: **colorless liquid**

Storage Conditions: **room temperature in the dark**

Test Article Receipt and Login: **09 May 2007**

Study Initiation: **30 July 2007**

Experimental Start Date: **01 August 2007**

Experimental Completion Date: **29 August 2007**

Laboratory Manager: **Emily W. Dakoulas, B.S.**

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## SUMMARY

The test article, 1-Chlorododecane, 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 and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation 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 the solubility of the test article and compatibility with the target cells. The test article formed a soluble and clear solution in ethanol at approximately 500 mg/mL, the highest concentration tested.

In the initial toxicity-mutation assay, the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 100 mg/mL and a 50 µL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate. In the initial toxicity-mutation assay, no positive mutagenic response was observed. Precipitate was observed at concentrations  $\geq 1500$  µg per plate for all tester strains in the presence and absence of metabolic activation. Precipitate was also observed at 500 µg per plate for TA98 in the presence of metabolic activation. No appreciable toxicity was observed. 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 response was observed. The dose levels tested were 50, 150, 500, 1500 and 5000 µg per plate. Precipitate was observed at concentrations  $\geq 1500$  µg per plate for all tester strains in the presence and absence of metabolic activation. No appreciable toxicity was observed.

Under the conditions of this study, test article **1-Chlorododecane** 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 amendments are included in Appendix II.

This study was conducted in compliance with the testing guidelines of the TSCA Health Effects Guideline, 40CFR Part 799.9510 (1999), the OECD Guideline 471 (1998) and the ICH (1996 and 1997).

## CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, 1-Chlorododecane (CAS No. 112-52-7; Batch No. N6227945; 97.4% purity), was received by BioReliance on 09 May 2007 and was assigned the code number AC01UM. The test article was characterized on the Material Safety Data Sheet as a colorless to pale yellow liquid. An expiration date of 29 June 2009 was provided by the Sponsor. Per the protocol, the test article should be stored at ambient temperature in the dark. Upon receipt, the test article was described as a colorless liquid and was stored at room temperature in the dark.

The Sponsor has determined the identity, strength, purity, composition or other characteristics to define the test article and the stability of the test article. Copies of the Certificate of Analysis and Stability Memo are included in Appendix IV. The Stability Memo indicated that the test article was stable through 24 April 2008.

The vehicle used to deliver 1-Chlorododecane to the test system was ethanol (EtOH, CAS No. 64-17-5, Lot No. B0512128, Exp. Date: November 2009), obtained from Acrōs Organics. Test article dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light.

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 the initial toxicity-mutation assay and the confirmatory mutagenicity assay are listed in the following table. All positive controls were diluted in dimethyl sulfoxide (DMSO) except for sodium azide, which was diluted in water. All subdivided solutions of positive control were stored at -5 to -30°C.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All <i>Salmonella</i> Strains	Rat	2-aminoanthracene (Aldrich Chemical Co., Inc.) Lot No. 12317CE Exp. Date 01-Feb-2009 CAS No. 613-13-8 Purity 99.9%	1.0
WP2 <i>uvrA</i>		10	
TA98	None	2-nitrofluorene (Aldrich Chemical Co., Inc.) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Sigma Chemical Co.) Lot No. 073K0119 Exp. Date 31-Jul-2008 CAS No. 26628-22-8 Purity 99.9%	1.0
TA1537		9-aminoacridine (Sigma Chemical Co.) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 <i>uvrA</i>		methyl methanesulfonate (Aldrich Chemical Co., Inc.) Lot No. 05713JD Exp. Date 15-Jan-2009 CAS No. 66-27-3 Purity 99.9%	1,000

To confirm the sterility of the test article, the highest test article dose levels used in the initial toxicity-mutation and confirmatory mutagenicity assays 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

For submission to Japanese regulatory agencies, additional information is included in Appendix III.

### 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 received from Dr. Bruce Ames' designated distributor, Discovery Partners International, San Diego, California. *E. coli* tester strains were received 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 basepair substitution mutations, rather than frameshift mutations (Green and Muriel, 1976).

Overnight cultures were prepared by inoculating from the appropriate master plate or from the appropriate frozen permanent stock into a vessel containing ~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 resting shaker/incubator at room temperature. The shaker/incubator was programmed to begin shaking at approximately 125 rpm at 37±2°C 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 approximately 10<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 induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The lot of S9 was prepared by and purchased 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 at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.

The S9 mix was prepared immediately before its use and contained 10% S9, 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. The Sham S9 mixture (Sham mix), containing 100 mM phosphate buffer at pH 7.4, was prepared immediately before its 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 to determine the vehicle. The test was conducted using water, dimethyl sulfoxide (DMSO) and ethanol (EtOH). The test article was tested to determine the vehicle, selected in order of preference, that permitted preparation of the highest soluble or workable stock concentration, up to 50 mg/mL for aqueous solvents and 500 mg/mL for organic solvents.

### **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. Five 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 plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983).

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 water for each 100 mL of minimal top agar. For the preparation of media and reagents, all references to water imply sterile, deionized water produced by the Milli-Q Reagent Water System. 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 with a code system that identified the test article, test phase, dose level, tester strain and activation, as described in detail in BioReliance's Standard Operating Procedures.

BioReliance

Study No. AC01UM.503.BTL

One-half (0.5) milliliter of S9 or Sham mix, 100 µL of tester strain and 50 µL of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45±2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test article aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 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 after the incubation period 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	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 greater than or equal to 90% of the plate.
6	Obscured by Particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
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 or equal to 10% of the revertant colony count (e.g., less than or equal to 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., greater 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 equal to or greater than 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 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.

## Criteria for a Valid Test

The following criteria must be met for the initial toxicity-mutation and the confirmatory mutagenicity assays 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:

Minicount Colony Counter (Imaging Products International), LIMS Labware Version 5, Configured Version 1.7 (BioReliance), PC Ames System (BioReliance), Excel 2003 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

### **Archives**

All raw data, the protocol and all reports for procedures performed at BioReliance will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years. The raw data, reports and other documents generated at facilities other than BioReliance will be archived per the contractual arrangements between the facility and the Sponsor.

### **Deviations**

No known deviations from the protocol or assay-method SOPs occurred during the conduct of this study.

## RESULTS AND DISCUSSION

### Solubility Test

Ethanol (EtOH) was selected as the solvent of choice based on the solubility of the test article and compatibility with the target cells. The test article formed a soluble and clear solution in ethanol at approximately 500 mg/mL, the highest concentration tested.

### Sterility Results

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

### Initial Toxicity-Mutation Assay

The results of the initial toxicity-mutation assay are presented in Tables 1 through 10 and summarized in Table 21. These data were generated in Experiment B1 (Initial Toxicity-Mutation Assay). In the initial toxicity-mutation assay, the maximum dose tested was 5000  $\mu\text{g}$  per plate; this dose was achieved using a concentration of 100 mg/mL and a 50  $\mu\text{L}$  plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000  $\mu\text{g}$  per plate. Precipitate was observed at concentrations  $\geq 1500$   $\mu\text{g}$  per plate for all tester strains in the presence and absence of metabolic activation. Precipitate was also observed at 500  $\mu\text{g}$  per plate for TA98 in the presence of metabolic activation. No appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000  $\mu\text{g}$  per plate.

In Experiment B1, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

### Confirmatory Mutagenicity Assay

The results of the confirmatory mutagenicity assay are presented in Tables 11 through 20 and summarized in Table 22. These data were generated in Experiment B2 (Confirmatory Mutagenicity Assay). The dose levels tested were 50, 150, 500, 1500 and 5000  $\mu\text{g}$  per plate. Precipitate was observed at concentrations  $\geq 1500$   $\mu\text{g}$  per plate for all tester strains in the presence and absence of metabolic activation. No appreciable toxicity was observed.

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

## 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-Chlorododecane**

did not cause a positive mutagenic response in either the presence or absence of Aroclor-induced rat liver S9.

## 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*<sup>+</sup> reversion in *Escherichia coli*, *Mutation Research* 38:3-32.

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

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United States Environmental Protection Agency (EPA), Health Effects Test Guideline, 40 CFR Part 799.9510 (TSCA Bacterial Reverse Mutation Test). 62 FR 43824, August 15, 1997, as amended at 64 FR 35079, June 30, 1999.

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

Bacterial Mutation Assay

Table 1

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA98 Cells Seeded : 1.8 X 10<sup>8</sup>  
 Liver Microsomes : None Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	21	1	20	2
	02	18	1		
1.5	01	14	1	19	6
	02	23	1		
5.0	01	20	1	20	1
	02	19	1		
15	01	16	1	15	1
	02	14	1		
50	01	20	1	20	1
	02	19	1		
150	01	C		21	--
	02	21	1		
500	01	13	1	17	6
	02	21	1		
1500	01	15	1NP	15	--
	02	C			
5000	01	17	1NP	17	1
	02	16	1NP		
Positive Control 2-nitrofluorene 1.0 µg per plate					
	01	157	1	161	5
	02	164	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced

4=Extremely reduced; 5=Absent; 6=Obscured by particulate

NP=Non-Interfering precipitate; IP=Interfering precipitate

C=Contaminated (The loss of these test article-treated plates does not invalidate the results since the remaining plate at each dose level and the remaining treated plates are also comparable to the negative control.)

Bacterial Mutation Assay

Table 2

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA98 Cells Seeded : 1.8 X 10<sup>8</sup>  
 Liver Microsomes : Rat liver S9 Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	17	1	20	4
	02	22	1		
1.5	01	31	1	22	13
	02	13	1		
5.0	01	28	1	24	6
	02	19	1		
15	01	25	1	22	4
	02	19	1		
50	01	28	1	31	4
	02	33	1		
150	01	27	1	22	7
	02	17	1		
500	01	26	1NP	29	4
	02	31	1NP		
1500	01	26	1NP	27	1
	02	27	1NP		
5000	01	26	1NP	20	8
	02	14	1NP		
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	491	1	467	34
	02	443	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 3

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA100 Cells Seeded : 1.8 X 10<sup>8</sup>  
 Liver Microsomes : None Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	176	1	167	13
	02	158	1		
1.5	01	148	1	144	6
	02	140	1		
5.0	01	156	1	156	--
	02	C			
15	01	144	1	152	11
	02	159	1		
50	01	128	1	128	--
	02	C			
150	01	107	1	104	4
	02	101	1		
500	01	131	1	136	6
	02	140	1		
1500	01	123	1NP	122	1
	02	121	1NP		
5000	01	76	1NP	85	12
	02	93	1NP		
Positive Control sodium azide 1.0 µg per plate					
	01	583	1		
	02	447	1	515	96

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate  
 C=Contaminated (The loss of these test article-treated plates does not invalidate the results since the remaining plate at each dose level and the remaining treated plates are also comparable to the negative control.)

Bacterial Mutation Assay

Table 4

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA100 Cells Seeded : 1.8 X 10<sup>8</sup>  
 Liver Microsomes : Rat liver S9 Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	154	1	158	6
	02	162	1		
1.5	01	156	1	157	1
	02	158	1		
5.0	01	154	1	156	3
	02	158	1		
15	01	155	1	172	24
	02	189	1		
50	01	152	1	171	27
	02	190	1		
150	01	175	1	174	1
	02	173	1		
500	01	146	1	158	16
	02	169	1		
1500	01	129	1NP	148	26
	02	166	1NP		
5000	01	125	1NP	149	33
	02	172	1NP		
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	442	1	557	162
	02	671	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 5

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA1535 Cells Seeded : 2.4 X 10<sup>8</sup>  
 Liver Microsomes : None Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	15	1	19	5
	02	22	1		
1.5	01	20	1	18	3
	02	16	1		
5.0	01	19	1	17	3
	02	15	1		
15	01	17	1	17	0
	02	17	1		
50	01	23	1	23	0
	02	23	1		
150	01	18	1	20	3
	02	22	1		
500	01	21	1	15	8
	02	9	1		
1500	01	14	1NP	17	4
	02	19	1NP		
5000	01	8	1NP	13	6
	02	17	1NP		
Positive Control sodium azide 1.0 µg per plate					
	01	335	1	376	58
	02	417	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 6

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA1535 Cells Seeded : 2.4 X 10<sup>8</sup>  
 Liver Microsomes : Rat liver S9 Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	13	1	13	0
	02	13	1		
1.5	01	26	1	26	0
	02	26	1		
5.0	01	18	1	16	4
	02	13	1		
15	01	22	1	19	4
	02	16	1		
50	01	20	1	22	2
	02	23	1		
150	01	16	1	14	3
	02	12	1		
500	01	18	1	17	2
	02	15	1		
1500	01	17	1NP	20	4
	02	22	1NP		
5000	01	16	1NP	14	3
	02	12	1NP		
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	61	1	68	9
	02	74	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 7

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL      Experiment No : B1  
 Strain : TA1537      Cells Seeded : 2.0 X 10<sup>8</sup>  
 Liver Microsomes : None      Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	6	1	7	1
	02	7	1		
1.5	01	5	1	6	1
	02	7	1		
5.0	01	3	1	4	1
	02	4	1		
15	01	8	1	7	2
	02	5	1		
50	01	5	1	6	1
	02	7	1		
150	01	5	1	7	2
	02	8	1		
500	01	8	1	6	4
	02	3	1		
1500	01	4	1NP	5	1
	02	5	1NP		
5000	01	9	1NP	7	3
	02	5	1NP		
Positive Control 9-aminoacridine 75 µg per plate					
	01	1226	1	1174	74
	02	1121	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 8

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : TA1537 Cells Seeded : 2.0 X 10<sup>8</sup>  
 Liver Microsomes : Rat liver S9 Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	7	1	8	1
	02	8	1		
1.5	01	6	1	7	1
	02	7	1		
5.0	01	10	1	9	2
	02	7	1		
15	01	11	1	9	4
	02	6	1		
50	01	3	1	3	0
	02	3	1		
150	01	8	1	7	1
	02	6	1		
500	01	5	1	6	1
	02	6	1		
1500	01	4	1NP	7	4
	02	9	1NP		
5000	01	5	1NP	5	1
	02	4	1NP		
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	50	1	43	11
	02	35	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 9

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL      Experiment No : B1  
 Strain : WP2 *uvrA*      Cells Seeded : 2.9 X 10<sup>8</sup>  
 Liver Microsomes : None      Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	48	1	43	7
	02	38	1		
1.5	01	43	1	44	1
	02	45	1		
5.0	01	37	1	38	1
	02	39	1		
15	01	48	1	44	6
	02	40	1		
50	01	53	1	53	1
	02	52	1		
150	01	51	1	44	11
	02	36	1		
500	01	36	1	43	10
	02	50	1		
1500	01	34	1NP	35	1
	02	36	1NP		
5000	01	30	1NP	36	8
	02	42	1NP		
Positive Control methyl methanesulfonate 1000 µg per plate					
	01	290	1	296	8
	02	302	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 10

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL Experiment No : B1  
 Strain : WP2 *uvrA* Cells Seeded : 2.9 X 10<sup>8</sup>  
 Liver Microsomes : Rat liver S9 Date Plated : 1 Aug 2007  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	47	1	52	7
	02	57	1		
1.5	01	37	1	40	4
	02	43	1		
5.0	01	41	1	40	2
	02	38	1		
15	01	55	1	44	16
	02	33	1		
50	01	38	1	40	3
	02	42	1		
150	01	44	1	42	4
	02	39	1		
500	01	36	1	46	14
	02	56	1		
1500	01	33	1NP	38	6
	02	42	1		
5000	01	36	1NP	35	2
	02	33	1NP		
Positive Control 2-aminoanthracene 10 µg per plate					
	01	295	1		
	02	260	1	278	25

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced  
 4=Extremely reduced; 5=Absent; 6=Obscured by particulate  
 NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 11

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA98  
 Liver Microsomes : None  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL  
 Experiment No : B2  
 Cells Seeded : 1.7 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	19	1		
	02	10	1		
	03	10	1	13	5
50	01	11	1		
	02	8	1		
	03	14	1	11	3
150	01	11	1		
	02	18	1		
	03	10	1	13	4
500	01	14	1		
	02	17	1		
	03	18	1	16	2
1500	01	13	1NP		
	02	9	1NP		
	03	12	1NP	11	2
5000	01	8	1NP		
	02	19	1NP		
	03	8	1NP	12	6
Positive Control	2-nitrofluorene 1.0 µg per plate				
	01	150	1		
	02	156	1		
	03	146	1	151	5

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 12

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA98  
 Liver Microsomes : Rat Liver S9  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Experiment No : B2  
 Cells Seeded : 1.7 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	24	1		
	02	15	1		
	03	18	1	19	5
50	01	21	1		
	02	24	1		
	03	24	1	23	2
150	01	19	1		
	02	19	1		
	03	16	1	18	2
500	01	19	1		
	02	19	1		
	03	21	1	20	1
1500	01	9	1NP		
	02	11	1NP		
	03	21	1NP	14	6
5000	01	16	1NP		
	02	13	1NP		
	03	11	1NP	13	3
Positive Control	2-aminoanthracene 1.0 µg per plate				
	01	471	1		
	02	346	1		
	03	454	1	424	68

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 13

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA100  
 Liver Microsomes : None  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL  
 Experiment No : B2  
 Cells Seeded : 1.5 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	135	1	137	10
	02	128	1		
	03	148	1		
50	01	148	1	142	6
	02	136	1		
	03	142	1		
150	01	126	1	117	14
	02	124	1		
	03	100	1		
500	01	139	1	132	8
	02	132	1		
	03	124	1		
1500	01	137	1NP	135	10
	02	124	1NP		
	03	144	1NP		
5000	01	160	1NP	155	9
	02	161	1NP		
	03	145	1NP		
Positive Control	sodium azide 1.0 µg per plate				
	01	540	1	495	41
	02	485	1		
	03	459	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 14

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA100 Experiment No : B2  
 Liver Microsomes : Rat Liver S9 Cells Seeded :  $1.5 \times 10^8$   
 Vehicle : ethanol Date Plated : 17 Aug 2007  
 Plating Aliquot : 50  $\mu$ L

Concentration $\mu$ g per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	168	1		
	02	183	1		
	03	190	1	180	11
50	01	122	1		
	02	156	1		
	03	148	1	142	18
150	01	108	1		
	02	165	1		
	03	174	1	149	36
500	01	177	1		
	02	169	1		
	03	199	1	182	16
1500	01	210	1NP		
	02	169	1NP		
	03	189	1NP	189	21
5000	01	152	1NP		
	02	175	1NP		
	03	176	1NP	168	14
Positive Control	2-aminoanthracene 1.0 $\mu$ g per plate				
	01	954	1		
	02	853	1		
	03	669	1	825	145

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 15

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA1535  
 Liver Microsomes : None  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL  
 Experiment No : B2  
 Cells Seeded : 1.3 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	19	1		
	02	17	1		
	03	19	1	18	1
50	01	18	1		
	02	16	1		
	03	22	1	19	3
150	01	17	1		
	02	14	1		
	03	11	1	14	3
500	01	22	1		
	02	21	1		
	03	23	1	22	1
1500	01	15	1NP		
	02	24	1NP		
	03	20	1NP	20	5
5000	01	18	1NP		
	02	21	1NP		
	03	19	1NP	19	2
Positive Control	sodium azide 1.0 µg per plate				
	01	362	1		
	02	317	1		
	03	427	1	369	55

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 16

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA1535 Experiment No : B2  
 Liver Microsomes : Rat Liver S9 Cells Seeded : 1.3 X 10<sup>8</sup>  
 Vehicle : ethanol Date Plated : 17 Aug 2007  
 Plating Aliquot : 50 µL

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	20	1		
	02	27	1		
	03	36	1	28	8
50	01	18	1		
	02	21	1		
	03	18	1	19	2
150	01	30	1		
	02	29	1		
	03	20	1	26	6
500	01	15	1		
	02	18	1		
	03	22	1	18	4
1500	01	25	1NP		
	02	24	1NP		
	03	25	1NP	25	1
5000	01	18	1NP		
	02	12	1NP		
	03	18	1NP	16	3
Positive Control	2-aminoanthracene 1.0 µg per plate				
	01	85	1		
	02	122	1		
	03	94	1	100	19

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 17

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA1537  
 Liver Microsomes : None  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Experiment No : B2  
 Cells Seeded : 1.7 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	6	1		
	02	5	1		
	03	4	1	5	1
50	01	2	1		
	02	4	1		
	03	5	1	4	2
150	01	8	1		
	02	9	1		
	03	3	1	7	3
500	01	5	1		
	02	5	1		
	03	6	1	5	1
1500	01	9	1NP		
	02	7	1NP		
	03	6	1NP	7	2
5000	01	12	1NP		
	02	3	1NP		
	03	4	1NP	6	5
Positive Control	9-aminoacridine 75 µg per plate				
	01	924	1		
	02	864	1		
	03	1062	1	950	102

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 18

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : TA1537  
 Liver Microsomes : Rat Liver S9  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL

Experiment No : B2  
 Cells Seeded : 1.7 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	5	1		
	02	6	1		
	03	8	1	6	2
50	01	7	1		
	02	9	1		
	03	8	1	8	1
150	01	5	1		
	02	10	1		
	03	10	1	8	3
500	01	8	1		
	02	6	1		
	03	8	1	7	1
1500	01	5	1NP		
	02	6	1NP		
	03	10	1NP	7	3
5000	01	5	1NP		
	02	5	1NP		
	03	5	1NP	5	0
Positive Control	2-aminoanthracene 1.0 µg per plate				
	01	60	1		
	02	76	1		
	03	62	1	66	9

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay

Table 19

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : WP2 uvrA Experiment No : B2  
 Liver Microsomes : None Cells Seeded :  $2.3 \times 10^8$   
 Vehicle : ethanol Date Plated : 17 Aug 2007  
 Plating Aliquot : 50  $\mu$ L

Concentration $\mu$ g per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	50	1		
	02	41	1		
	03	44	1	45	5
50	01	48	1		
	02	52	1		
	03	40	1	47	6
150	01	40	1		
	02	40	1		
	03	34	1	38	3
500	01	49	1		
	02	44	1		
	03	41	1	45	4
1500	01	44	1NP		
	02	45	1NP		
	03	44	1NP	44	1
5000	01	33	1NP		
	02	21	1NP		
	03	35	1NP	30	8
Positive Control	methyl methanesulfonate 1000 $\mu$ g per plate				
	01	281	1		
	02	307	1		
	03	379	1	322	51

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced;  
 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate;  
 IP=Interfering precipitate

Bacterial Mutation Assay

Table 20

Test Article Id : 1-Chlorododecane  
 Study Number : AC01UM.503.BTL  
 Strain : WP2 uvrA  
 Liver Microsomes : Rat Liver S9  
 Vehicle : ethanol  
 Plating Aliquot : 50 µL  
 Experiment No : B2  
 Cells Seeded : 2.3 x 10<sup>8</sup>  
 Date Plated : 17 Aug 2007

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	47	1	50	7
	02	58	1		
	03	45	1		
50	01	46	1	42	4
	02	38	1		
	03	41	1		
150	01	37	1	41	6
	02	39	1		
	03	48	1		
500	01	26	1	33	8
	02	33	1		
	03	41	1		
1500	01	37	1NP	41	4
	02	45	1NP		
	03	42	1NP		
5000	01	23	1NP	29	6
	02	32	1NP		
	03	33	1NP		
Positive Control	2-aminoanthracene 10 µg per plate				
	01	215	1	239	33
	02	276	1		
	03	226	1		

Background Lawn Code

1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent; 6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay  
Summary of Results - Initial Toxicity-Mutation Assay

Table 21

Test Article Id : 1-Chlorododecane  
Study Number : AC01UM.503.BTL Experiment No : B1

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Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98		TA100		TA1535		TA1537		WP2 <i>uvrA</i>	
Vehicle	20 ± 2	167 ± 13	19 ± 5	7 ± 1	43 ± 7					
1.5	19 ± 6	144 ± 6	18 ± 3	6 ± 1	44 ± 1					
5.0	20 ± 1	156 ± --	17 ± 3	4 ± 1	38 ± 1					
15	15 ± 1	152 ± 11	17 ± 0	7 ± 2	44 ± 6					
50	20 ± 1	128 ± --	23 ± 0	6 ± 1	53 ± 1					
150	21 ± --	104 ± 4	20 ± 3	7 ± 2	44 ± 11					
500	17 ± 6	136 ± 6	15 ± 8	6 ± 4	43 ± 10					
1500	15 ± --	122 ± 1	17 ± 4	5 ± 1	35 ± 1					
5000	17 ± 1	85 ± 12	13 ± 6	7 ± 3	36 ± 8					
Positive	161 ± 5	515 ± 96	376 ± 58	1174 ± 74	296 ± 8					

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98		TA100		TA1535		TA1537		WP2 <i>uvrA</i>	
Vehicle	20 ± 4	158 ± 6	13 ± 0	8 ± 1	52 ± 7					
1.5	22 ± 13	157 ± 1	26 ± 0	7 ± 1	40 ± 4					
5.0	24 ± 6	156 ± 3	16 ± 4	9 ± 2	40 ± 2					
15	22 ± 4	172 ± 24	19 ± 4	9 ± 4	44 ± 16					
50	31 ± 4	171 ± 27	22 ± 2	3 ± 0	40 ± 3					
150	22 ± 7	174 ± 1	14 ± 3	7 ± 1	42 ± 4					
500	29 ± 4	158 ± 16	17 ± 2	6 ± 1	46 ± 14					
1500	27 ± 1	148 ± 26	20 ± 4	7 ± 4	38 ± 6					
5000	20 ± 8	149 ± 33	14 ± 3	5 ± 1	35 ± 2					
Positive	467 ± 34	557 ± 162	68 ± 9	43 ± 11	278 ± 25					

Vehicle = Vehicle Control

Positive = Positive Control (50 µL plating aliquot)

Plating aliquot: 50 µL

Bacterial Mutation Assay  
Summary of Results - Confirmatory Mutagenicity Assay

Table 22

Test Article Id : 1-Chlorododecane

Study Number : AC01UM.503.BTL

Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes : None

Dose (µg/plate)	TA98		TA100		TA1535		TA1537		WP2 <i>uvrA</i>	
Vehicle	13 ± 5	137 ± 10	18 ± 1	5 ± 1	45 ± 5					
50	11 ± 3	142 ± 6	19 ± 3	4 ± 2	47 ± 6					
150	13 ± 4	117 ± 14	14 ± 3	7 ± 3	38 ± 3					
500	16 ± 2	132 ± 8	22 ± 1	5 ± 1	45 ± 4					
1500	11 ± 2	135 ± 10	20 ± 5	7 ± 2	44 ± 1					
5000	12 ± 6	155 ± 9	19 ± 2	6 ± 5	30 ± 8					
Positive	151 ± 5	495 ± 41	369 ± 55	950 ± 102	322 ± 51					

Liver Microsomes : Rat Liver S9

Dose (µg/plate)	TA98		TA100		TA1535		TA1537		WP2 <i>uvrA</i>	
Vehicle	19 ± 5	180 ± 11	28 ± 8	6 ± 2	50 ± 7					
50	23 ± 2	142 ± 18	19 ± 2	8 ± 1	42 ± 4					
150	18 ± 2	149 ± 36	26 ± 6	8 ± 3	41 ± 6					
500	20 ± 1	182 ± 16	18 ± 4	7 ± 1	33 ± 8					
1500	14 ± 6	189 ± 21	25 ± 1	7 ± 3	41 ± 4					
5000	13 ± 3	168 ± 14	16 ± 3	5 ± 0	29 ± 6					
Positive	424 ± 68	825 ± 145	100 ± 19	66 ± 9	239 ± 33					

Vehicle = Vehicle Control

Positive = Positive Control (50 µL plating aliquot)

Plating aliquot: 50 µL

**APPENDIX I**

Historical Control Data

Historical Negative and Positive Control Values

2004 – 2006

revertants per plate

Strain	Control	Activation							
		None				Rat Liver			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA98	Neg	18	6	5	56	27	8	6	72
	Pos	184	129	30	1812	666	434	61	2669
TA100	Neg	132	31	52	632	141	29	67	267
	Pos	545	147	112	4349	828	410	168	2652
TA1535	Neg	20	7	4	49	16	5	4	45
	Pos	376	131	44	998	114	60	24	985
TA1537	Neg	7	3	1	20	8	3	1	22
	Pos	692	343	14	2216	101	97	13	1297
WP2 <i>uvrA</i>	Neg	18	7	5	53	19	7	4	59
	Pos	151	98	28	892	394	268	29	1296

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 and Amendments**

QA Reviewed

~~AC 20 July 2007~~  
Init. Date

PROTOCOL AMENDMENT 1

Sponsor: Lonza, Inc

Test Article I.D.: 1-Chlorododecane

BioReliance Study No.: AC01UM.503.BTL

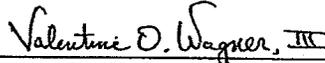
Protocol Title: Bacterial Reverse Mutation Assay of 1-Chlorododecane

1. **LOCATION:** Pages 2 to 12, BioReliance Study Number

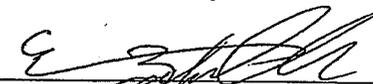
**AMENDMENT:** The BioReliance Study number should be AC01UM.503.BTL rather than ACX01UM.503.BTL.

**REASON FOR THE AMENDMENT:** Error during protocol preparation

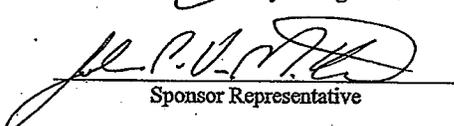
**APPROVALS:**

  
\_\_\_\_\_  
BioReliance Study Director

30 Jul 2007  
Date

  
\_\_\_\_\_  
BioReliance Study Management

30 July 2007  
Date

  
\_\_\_\_\_  
Sponsor Representative

24 Aug 2007  
Date

QA Reviewed

PROTOCOL AMENDMENT 2

*MMA* 21 Aug 2007  
Init. Date

Sponsor: Lonza, Inc

Test Article ID.: 1-Chlorododecane

BioReliance Study No.: AC01UM.503.BTL

Protocol Title: Bacterial Reverse Mutation Assay of 1-Chlorododecane

1. LOCATION: Page 4, §7.3; Confirmatory Mutagenicity Assay

AMENDMENT: Add the following statement: The dose levels for the confirmatory mutagenicity assay will be 5000, 1500, 500, 150 and 50 µg per plate.

REASON FOR THE AMENDMENT: To specify the dose levels for the confirmatory mutagenicity assay on the basis of the precipitation and toxicity profiles in the initial toxicity-mutation assay. In the initial toxicity-mutation assay, precipitate was observed beginning at 500, 1500 or at 5000 µg per plate. No toxicity was observed.

APPROVALS:

*Valentine O. Wagner, III*  
\_\_\_\_\_  
BioReliance Study Director

*14 Aug 2007*  
\_\_\_\_\_  
Date

*Ramadevi Aul*  
\_\_\_\_\_  
BioReliance Study Management

*14 Aug 2007*  
\_\_\_\_\_  
Date

*J. P. V. [Signature]*  
\_\_\_\_\_  
Sponsor Representative

*23 Aug 2007*  
\_\_\_\_\_  
Date

QA Reviewed

Received by RA/OA 30 JUL 2007

ACI 30 Jul 2007  
Init. Date

BioReliance Study Number: AC01UM.503.BTL

**Bacterial Reverse Mutation Assay of 1-Chlorododecane**

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: Lonza, Inc  
2.2 Address: 90 Boroline Road  
Allendale, NJ 07401  
2.3 Representative: John P. Van Miller, Ph.D., DABT  
Toxicology Regulatory Services, Inc.  
Phone: 434-977-5957  
Fax: 434-977-0899  
Email: jvanmiller@toxregserv.com

3.0 TEST AND CONTROL ARTICLES

3.1 Test Article Name: 1-Chlorododecane  
CAS No.: 112-52-7  
Storage Temperature: Ambient  
Storage Parameters: Unless otherwise indicated, all test articles will be stored in the dark and solids will be stored with desiccant.  
Purity: An adjustment for purity or active ingredient will not be made unless indicated by the Sponsor.  
Molecular Weight: 204.783  
Batch No.: N6227945  
Appearance: To be documented in the study records  
Expiration Date: To be provided by the Sponsor

Protocol SPGT503 17-Jul-2007 1 of 12

BioReliance Study Number: ACX01UM.503.BTL

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 or dosing solutions. 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).

3.4 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.

3.5 Residual Test Article and Dosing Preparations

Dosing preparations 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

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 02-Aug-2007

5.2 Proposed Experimental Completion Date: 07-Sept-2007

5.3 Proposed Report Date: 21-Sept-2007

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## 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 received directly from Dr. Bruce Ames, University of California, Berkeley or a vendor authorized by his laboratory. The *E. coli* tester strain was received from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (United Kingdom).

## 7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

### 7.1 Solubility Determination

As needed, a solubility determination will be conducted to determine the maximum soluble concentration or workable suspension as indicated below. Vehicles compatible with this test system, in order of preference, include but are not limited to deionized water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5) and acetone (CAS 67-64-1). The vehicle of choice will be the solvent, selected in order of preference, which permits preparation of the highest workable or soluble stock concentration, up to 50 mg/mL for aqueous vehicles and up to 500 mg/mL for organic vehicles. Based on the molecular weight of the test article, the solvents to be tested and the dose to be achieved in the assay, alternate stock concentrations may be tested, as needed.

### 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 for the confirmatory mutagenicity assay and the selected doses will be documented in an amendment to the protocol.**

### 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 plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983). 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 (1998) and ICH Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (1997).

## 7.5 Controls

## 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
WP2 <i>uvrA</i>			10
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

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 will be batch prepared and stored frozen at -60°C or colder until used. Each batch of S9 homogenate 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  $\beta$ -nicotinamide-adenine dinucleotide phosphate, 8 mM  $MgCl_2$  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

Overnight cultures will be inoculated from the appropriate master plate or from the appropriate frozen stock. To ensure that cultures are harvested in late log phase, the length of incubation will be controlled and monitored. At the end of the working day, each inoculated flask will be placed in a resting shaker/incubator at room temperature. The shaker/incubator will be programmed to begin shaking at approximately 125 rpm at 37±2°C approximately 12 hours before the anticipated time of harvest.

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, 100 µL of tester strain and 50 µL of vehicle, test article dilution or positive control will be added to 2.0 mL of molten selective top agar at 45±2°C. When necessary to achieve the target concentration or eliminate toxic vehicle effects, aliquots of other than 50 µL of test article or vehicle or positive control will be plated. When plating untreated controls, the addition of test article, vehicle and positive control will be omitted. The mixture will be vortex mixed and overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay has solidified, the plates will be inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that are not counted immediately following the incubation period will be stored at 2-8°C and counted within 16 days of dosing.

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 assays, 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 not limited to the following:

Minicount Colony Counter (Imaging Products International), Oracle (Oracle Corporation), LIMS System (BioReliance), PC Ames System (BioReliance), Excel 2003 (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:

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 number 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 Titers

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 be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. Unless alternate arrangements are made, the report will be initially issued as a QA-audited draft. After receipt of the Sponsor's comments a final report will be issued. The report will include:

- Test article: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.
- Solvent/Vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- Strains: strains used; number of cells/mL per culture; strain characteristics.
- Test conditions: amount of test article per plate with rationale for dose selection and number of plates per concentration; media used; type and composition of metabolic activation system, including acceptability criteria; treatment procedures.
- Results: signs of toxicity; signs of precipitation; individual plate counts; the mean number of revertant colonies per plate and standard deviation; dose-response relationship, if any; statistical analysis, if any; concurrent negative and positive control data means and standard deviations.
- Discussion of results
- Conclusion
- Appendices: Historical Control Data (negative and positive controls with ranges, means and standard deviations), copy of protocol and any amendment, 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

If an electronic copy of the protocol, the report or another study document is provided by BioReliance, the executed paper document is considered the official master document. If there is a discrepancy between an electronic copy and the corresponding master document, the master document will be considered the official document.

#### 11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports for procedures performed at BioReliance will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years. The raw data, reports and other documents generated at facilities other than BioReliance will be archived per the contractual arrangements between the facility and the Sponsor.

#### 12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with TSCA Health Effects Test Guideline, 40 CFR Part 799.9510 (TSCA Bacterial Reverse Mutation Test), OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998 and with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996 and 1997).

This study will be conducted in compliance with the provisions of the US FDA GLP Regulations 21 CFR 58, the U.S. EPA GLP Standard 40 CFR 792 and the OECD Principles of Good Laboratory Practice. Multisite activities (if any) will be conducted per SOP ODQP2411. Changes to the protocol after Study Director signature will be made via protocol amendment. An in-process phase, the raw data, and report(s) will be inspected per the Standard Operating Procedures (SOPs) of BioReliance by the Quality Assurance Unit of BioReliance for compliance with GLPs, the SOPs of BioReliance and the study protocol. At least one, study-specific, in-process inspection will be performed for this study. A signed QA statement will be included in the final report. This statement will list the study-specific phases inspected, the dates of each inspection, and the dates the results of each inspection were reported to the Study Director and the Study Director's management. In addition, a signed GLP compliance statement will be included in the final report. This statement will cite the GLP guideline(s) with which the study is compliant and any exceptions to this compliance, if applicable, including the omission of characterization or stability analyses of the test article or its mixtures.

Raw data, the protocol and reports generated at facilities other than BioReliance will or will not be QA audited per the contractual arrangements between that facility and the Sponsor.

BioReliance Study Number: ACX01UM.503.BTL

Alterations of this protocol may be made as the study progresses. All protocol modifications and rationale for the change(s) will be documented, signed, dated and approved by the Study Director, BioReliance QA and the Sponsor/Sponsor Monitor. All protocol amendments will be delivered to the Sponsor and all Principal Investigators (if any) via mail, electronic file transfer or fax transmission, as well as internally at the Test Facility, on or as close as possible to the effective date of the amendment.

Deviations from the protocol (i.e., unplanned changes) will be documented in a deviation report. A deviation report will be signed by the Study Director and BioReliance QA. All deviations will be identified in the study 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.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202, April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. Federal Register 62:16026-16030, November 21, 1997.

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.

Maron, D.M. and Ames, B.N. (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, published by OECD, Paris, February 1998.

Protocol SPGT503

17-Jul-2007

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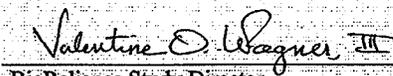
BioReliance Study Number: ACX01UM.503.BTL

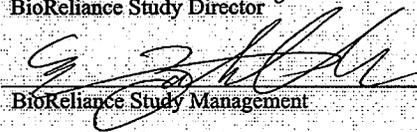
United States Environmental Protection Agency (EPA), Health Effects Test Guideline, 40 CFR Part 799.9510 (TSCA Bacterial Reverse Mutation Test). 62 FR 43824, August 15, 1997, as amended at 64 FR 35079, June 30, 1999.

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.

14.0 APPROVAL

  
Sponsor Representative 7/19/07  
Date

  
Valentine D. Wagner, III  
BioReliance Study Director 30 Jul 2007  
Date

  
BioReliance Study Management 30 July 2007  
Date

## **APPENDIX III**

### **Information for Japanese Regulatory Agencies**

## Report of Results of Reverse-Mutation Assay in Bacteria

### 1. Tester Strains

#### (1) Procurement

Strain	Obtained from	Date obtained	Date inspected the strain lot in storage
TA98	Dr. Bruce Ames' designated distributor, Discovery Partners International, San Diego, California	13 November 2002; 16 January 2004 (TA100 only)	The genetic markers for each culture are confirmed on the day of use
TA100			
TA1535			
TA1537			
TA1538			
TA97			
TA102			
WP2 <i>uvrA</i>	National Collection of Industrial and Marine Bacteria Aberdeen, Scotland	1 July 1987	
WP2 <i>uvrA</i> (pKM101)			
WP2 (pKM101)		19 February 1993	

#### (2) Storage

Freezing method	Large quantity	
Storage temperature	-60°C or colder	
Composition	Bacterial suspension	1.0 mL
	DMSO	0.09 mL

## 2. S9 Mix

### (1) Source, Storage Temperature, etc. of S9

Purchased from Moltox	Prepared on	Used in Experiment No.	
	18 April 2007 (Lot 2143)	B1 and B2	
Storage temperature	-60°C or colder	Name and model of storage apparatus	So-Low, Model PR120-9

### (2) Preparation of S9

Animal used		Inducing substance	
Species, Strain	Rattus norvegicus, Sprague Dawley	Name	Aroclor 1254
Sex	Male	Administration method	intraperitoneal
Age (in weeks)	Unknown (Lot 2143)	Administration period and amount (g/kg-weight)	single dose at 0.5 gm/kg body weight, 5 days prior to sacrifice
Weight	Unknown (Lot 2143)		

### 3. Preparation of Test Substance Solution

Solvent used			
Name	Manufacturer	Lot No.	Grade and/or Purity (%)
Ethanol (EtOH) (CAS No. 64-17-5)	Acrōs Organics	B0512128	99.9%
Stability of test substance in the solvent	Unknown		
Method of suspension when test substance is difficult to dissolve	Not applicable		

#### 4. Conditions of Pre-culture

Nutrient broth	Name	Manufacturer	Lot No.
	Oxoid Nutrient Broth No. 2	Oxoid Ltd.	464616
Period of pre-culture	12±2 hours		
Storage time and temp. from inoculation to beginning of shaking culture	<5 hours at ambient temperature		
Storage time and temp. from end of culture to use for test	<12 hours at 2-8°C		
Model and manufacturer of shaker	New Brunswick Scientific, model G-24		
Method of shaking (shaking type, speed, etc.)	Rotary (125 rev/min.)		
Culture vessel (shape, capacity)	shape: cylinder, 200 mL		
Culture volume	50 mL		
Volume of inoculum	1 colony		

5. Agar Plate Medium

(1) Top agar

Agar	Name	BBL Select
	Manufacturer	Becton Dickinson
	Lot No.	6332417 and 7078979

(2) Minimum Glucose Agar

Made in-house	Agar	Name		BBL Select
		Manufacturer		Becton Dickinson
		Lot No.		6332417 and 7078979
		Batch No.	Preparation Date	Used in Experiment No.
	16545	26 July 2007	B1	
	16554	30 July 2007		
	16582	16 August 2007	B2	
	Volume of agar plate medium		25 mL	

6. Test Results - Judgement of the results

Judgement	Negative
Reason for judgement and referential matters:	
No positive mutagenic response was observed with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9.	

Referential matters

The vehicle and positive control values indicate that all tester strains were functioning correctly and were capable of detecting a mutagen.
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**APPENDIX IV**

**Certificate of Analysis and Stability Memo**

# CERTIFICATE OF ANALYSIS

**Product:** 1-Chlorododecane

**Batch #:** N6227945

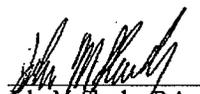
**PSL Reference No.:** 070416-3R

**Date of Analysis:** June 29, 2007

**Result:**

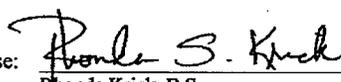
1-Chlorododecane 97.42%

Approval:

  
John M. Sheehy, B.A.,  
Analytical Services  
Product Safety Labs

8/16/07  
Date

QA Release:

  
Rhonda Krick, B.S.  
Quality Assurance  
Product Safety Labs

08/17/07  
Date

PSL GLP Study # 22018



# Toxicology Regulatory Services

May 8, 2008

Valentine (Skip) O. Wagner III, M.S.  
Study Director  
BioReliance  
9630 Medical Center Drive  
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Ramadevi Gudi, Ph.D.  
Study Director  
BioReliance  
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Re: 1-Chlorododecane: Confirmation of Stability

Dear Skip and Rama:

Following is a summary of the analyses that have been performed on behalf of Lonza Inc. in order to confirm stability of the referenced test article throughout the duration of testing conducted at BioReliance, e.g. Bacterial Reverse Mutation Assay and In Vitro Chromosomal Aberration Assay (Study Nos. AC01UM.503.BTL and AC01UM.331.BTL, respectively).

Characterization of the active ingredient in a test sample of 1-Chlorododecane was conducted at Eurofins|Product Safety Laboratories (E|PSL) as described in the table below.

E PSL Study No.	Test Article Identification	Batch No.	Date of Analysis	% Active Ingredient (mean)
22018	1-Chlorododecane (CAS No. 112-52-7)	#N6227945	June 29, 2007	97.42
24870	1-Chlorododecane (CAS No. 112-52-7)	#N6227945	April 24, 2008	98.8

The purity of the test sample remained within specification and no degradation of the material occurred between analyses. Based on these results, stability of 1-Chlorododecane (Batch No. N6227945) is confirmed for the period of June 29, 2007 through April 24, 2008.

Please contact me if you have any questions or require additional information.

Regards,

John P. Van Miller, Ph.D., DABT  
Toxicology Consultant to Lonza Inc.

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