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TRADE SECRETStudy Title

H-25170: Bacterial Reverse Mutation Test with an Independent Repeat Assay

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Study Completion Date

11 February 2002

Performing Laboratory

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Performing Laboratory Study Number

AA52XK.502001.BTL

Work Request Number

[REDACTED]

Service Code

[REDACTED]

### STATEMENT OF COMPLIANCE

Study No. AA52XK.502001.BTL was conducted in compliance with the U.S. FDA GLP Regulations as published in 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160, and 40 CFR 792, the UK GLP Compliance Programme, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test and control articles have not been determined by the testing facility. The control articles have been characterized as per the Certificates of Analysis on file with the testing facility.

The stability of the test and control articles has not been determined by the testing facility.

Analyses to determine the uniformity (as applicable), or concentration of the test and control mixtures were not performed by the testing facility. The Sponsor has indicated that they have not performed these analyses on the test substance mixtures.

The stability of the test and control articles in the test and control mixtures, respectively, has not been determined by the testing facility. The Sponsor has indicated that they have not performed stability analysis on the test substance mixtures.

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

11 Feb 2002  
Date

  
BioReliance Study Management

11 Feb 2002  
Date

## Quality Assurance Statement

**Study Title:** BACTERIAL REVERSE MUTATION ASSAY WITH AN INDEPENDENT  
REPEAT ASSAY

**Study Number:** AA52XK.502001.BTL

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

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is 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<br>Phase | 06-Dec-01 - 06-Dec-01 To Study Dir 06-Dec-01 To Mgmt 06-Dec-01<br>Protocol Review                             |
| Inspect On<br>Phase | 19-Dec-01 - 19-Dec-01 To Study Dir 19-Dec-01 To Mgmt 20-Dec-01<br>Test and/or control material administration |
| Inspect On<br>Phase | 30-Jan-02 - 01-Feb-02 To Study Dir 01-Feb-02 To Mgmt 07-Feb-02<br>Draft Report                                |
| Inspect On<br>Phase | 11-Feb-02 - 11-Feb-02 To Study Dir 11-Feb-02 To Mgmt 11-Feb-02<br>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.

  
\_\_\_\_\_  
Elahe Siadatpour, B.S.  
QUALITY ASSURANCE

11 Feb 2002  
DATE

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**CERTIFICATION**

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

Issued by Study Director:

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

11 Feb 2002  
Date

Approved by  
Study Monitor:

Maria Donner  
Maria Donner, Ph.D.  
Senior Research Scientist

08-Feb-2002  
Date

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STUDY INFORMATION

Substance Tested: { [REDACTED] }

Synonyms/Codes: { [REDACTED] }  
: H-25170  
: { [REDACTED] }

Submitter's Notebook Number(s): { [REDACTED] }

Haskell Number: 25170

CAS Registry Number: { [REDACTED] }

Composition: { [REDACTED] }

Purity: { [REDACTED] }

Known Impurities: { [REDACTED] }

Physical Characteristics: { [REDACTED] }

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

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

Study Initiated/Completed: December 05, 2001 / (see report cover page)

In-Life Initiated/Completed: December 07, 2001 / January 10, 2002



### SUMMARY

The test substance, H-25170, was tested in the Bacterial Reverse Mutation Test with an Independent Repeat 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 test was performed in two phases, using the plate incorporation method. The first phase, the preliminary toxicity test, was used to establish the dose-range for the mutagenicity test. The second phase, the mutagenicity test, (initial and independent repeat tests), was used to evaluate the mutagenic potential of the test substance.

Tetrahydrofuran (THF) was selected as the solvent of choice based on compatibility with the target cells and solubility of the test substance. The test substance was soluble but cloudy in tetrahydrofuran (THF) at a maximum concentration of approximately 200 mg/mL.

In the preliminary toxicity test, 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 test substance was a soluble but cloudy solution at 200 mg/mL and a soluble and clear solution from 0.27 to 133 mg/mL. Dose levels tested were 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg per plate. Precipitate was observed beginning at 67 or 100 µg per plate. No appreciable toxicity was observed. Based on the findings of the toxicity test, the maximum dose plated in the mutagenicity test was 5000 µg per plate.

In the mutagenicity test, no positive mutagenic response was observed. The dose levels tested were 15, 50, 150, 500, 1500 and 5000 µg per plate. Precipitate was observed beginning at 500 or 1500 µg per plate. No appreciable toxicity was observed.

The results of the Bacterial Reverse Mutation Test with an Independent Repeat Assay indicate that, under the conditions of this study, H-25170 did not cause a positive mutagenic response in either the presence or absence of Aroclor-induced rat liver S9.

## PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test substance 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 protocol is included in Appendix B.

The study was conducted in compliance with the testing guidelines of the ICH (1996 and 1997) and OECD ((1998).

## CHARACTERIZATION OF TEST AND CONTROL SUBSTANCES

The test substance, H-25170, was received by BioReliance on 03 December 2001 and was assigned the code number AA52XK. The test substance was characterized by the Sponsor as a [REDACTED] that should be stored at ambient temperature. An expiration date of 26 November 2004 was provided. Upon receipt, the test substance was described as a [REDACTED] and was stored at room temperature, protected from light and moisture. The identity, strength, purity, composition or other characteristics to define the test substance and the stability of the test substance have been determined by the Sponsor. The Sponsor indicated that BioReliance would not receive copies of these reports.

The vehicle used to deliver H-25170 to the test system was tetrahydrofuran (THF, CAS No. 109-99-9), Gold Label, 99.9%, purchased from Aldrich Chemical Company. Test substance dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light.

Positive controls plated concurrently with the mutagenicity test are listed below. All positive controls were diluted with dimethyl sulfoxide (DMSO) except sodium azide, which was diluted with water. All subdivided solutions of positive control were stored at -5 to -30°C.

| Strain                        | S9<br>Activation | Positive Control                                                                                                                 | Concentration<br>(µg/plate) |
|-------------------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| All <i>Salmonella</i> Strains | Rat              | 2-aminoanthracene<br>(Aldrich Chemical Co., Inc.)<br>Lot No. 09106PS<br>Exp. Date 14-Sep-2005<br>CAS No. 613-13-8<br>Purity >95% | 1.0                         |
| WP2 <i>uvrA</i>               |                  |                                                                                                                                  | 10                          |

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| Strain          | S9<br>Activation | Positive Control                                                                                                                                          | Concentration<br>(µg/plate) |
|-----------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| TA98            | None             | 2-nitrofluorene<br>(Aldrich Chemical Co., Inc.)<br>Lot No. 08707HS<br>Exp. Date 08-Mar-2006<br>CAS No. 607-57-8<br>Purity >98%                            | 1.0                         |
| TA100, TA1535   |                  | Sodium azide<br>(Sigma Chemical Co.)<br>Lot No. 098H0169<br>Exp. Date 05-Jan-2004<br>CAS No. 26628-22-8<br>Purity >99%                                    | 1.0                         |
| TA1537          |                  | 9-aminoacridine<br>(Sigma Chemical Co.)<br>Lot No. 106F06681<br>Exp. Date 18-Nov-2004<br>CAS No. 90-45-9<br>Purity >98%                                   | 75                          |
| WP2 <i>uvrA</i> |                  | Methyl methanesulfonate<br>(Aldrich Chemical Co., Inc.)<br>Lot No. 15526AO<br>Exp. Dates 08-Jan-2003 and<br>28-Nov-2003<br>CAS No. 66-27-3<br>Purity >99% | 1,000                       |

To confirm the sterility of the test substance, the highest test substance dose level used in the mutagenicity test was plated on selective agar with an aliquot volume equal to that used in the test. These plates were incubated under the same conditions as the test.

## MATERIALS AND METHODS

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

### 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 directly from Dr. Bruce Ames, University of California, Berkeley and *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 base-pair 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 tests on nutrient agar plates, and the data is on file but not presented in this report. The study was conducted to comply with OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), adopted July 1997 (published February 1998) and with the International Conference on Harmonisation of Technical Requirements of Registration of Pharmaceuticals for Human Use (1996 and 1997).

### 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 S9 was batch prepared and stored at -70°C or colder until used. Each bulk preparation of S9 was tested for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

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The S9 mix was prepared immediately before its use and contained 10% S9, 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. 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 select the vehicle. The test was conducted using water, dimethyl sulfoxide (DMSO), ethanol (EtOH), acetone, acetonitrile, tetrahydrofuran (THF), propylene glycol and polyethylene glycol. The test substance 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.

### **Preliminary Toxicity Test**

The preliminary toxicity test was used to establish the dose-range over which the test substance would be tested. Vehicle and ten dose levels of the test substance were plated, one plate 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. Dose levels tested were 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000  $\mu$ g per plate.

### **Mutagenicity Test**

The mutagenicity test (initial and independent repeat tests) was used to evaluate the mutagenic potential of the test substance. Six dose levels of test substance (15, 50, 150, 500, 1500 and 5000  $\mu$ g per plate) along with appropriate vehicle and positive controls were plated with TA98, TA100, TA1535, TA1537 and WP2 *uvrA* in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test substance, vehicle controls and positive controls were plated in triplicate.

### **Plating and Scoring Procedures**

The test system was exposed to the test substance 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  $\mu$ M 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 substance, test phase, dose level, tester strain, and activation, as described in detail in BioReliance's Standard Operating Procedures.

One-half (0.5) milliliter of S9 or Sham mix, 100  $\mu$ L of tester strain and 25  $\mu$ L of vehicle or test substance dilution were added to 2.0 mL of molten selective top agar at  $45\pm 2^\circ\text{C}$ . After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50  $\mu$ L aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at  $37\pm 2^\circ\text{C}$ . Plates that were not counted immediately following the incubation period were stored at  $2-8^\circ\text{C}$  until colony counting could be conducted (less than 10 days).

The condition of the bacterial background lawn was evaluated for evidence of test substance 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 below.

| 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 Precipitate | The background bacterial lawn cannot be accurately evaluated due to microscopic test substance precipitate.                                                                                                                                     |

| Code | Description                 | Characteristics                                                                                                                                                                                                                                                                            |
|------|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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 substance 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 substance. Data sets for tester strains TA1535 and TA1537 were 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. 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 is equal to or greater than 2.0-times the mean vehicle control value.

#### Criteria for a Valid Test

The following criteria must be met for the mutagenicity test 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 test 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

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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) A moderate reduction in the background lawn (background code of 3, 4 or 5). A copy of the Historical Negative and Positive Control Values is included in Appendix A.

#### **Archives**

All raw data, the protocol and all reports will be maintained according to Standard Operating Procedure [REDACTED] 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. Unused dosing solutions were disposed of following administration to the test system and all residual test substance will be disposed of following finalization of the report.

#### **Deviations**

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



## RESULTS AND DISCUSSION

### Solubility Test

Tetrahydrofuran (THF) was selected as the solvent of choice based on compatibility with the target cells and solubility of the test substance. The test substance was soluble but cloudy in tetrahydrofuran (THF) at a maximum concentration of approximately 200 mg/mL.

### Preliminary Toxicity Test

The results of the preliminary toxicity test are presented in Tables 1 through 5. These data were generated in Experiment A1. In the preliminary toxicity test, 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 test substance was a soluble but cloudy solution at 200 mg/mL and a soluble and clear solution from 0.27 to 133 mg/mL. Dose levels tested were 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg per plate. Precipitate was observed beginning at 67 or 100 µg per plate. No appreciable toxicity was observed. Based on the findings of the toxicity test, the maximum dose plated in the mutagenicity test was 5000 µg per plate.

### Mutagenicity Test

The results of the mutagenicity test are presented in Tables 6 through 25 and summarized in Tables 26 and 27. These data were generated in Experiments B1 and B2. Dose levels tested were 15, 50, 150, 500, 1500 and 5000 µg per plate. Precipitate was observed beginning at 500 or 1500 µg per plate. No appreciable toxicity was observed.

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

In Experiment B2 (Independent Repeat Test), 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 Test with an Independent Repeat Assay indicate that, under the conditions of this study, H-25170 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+ 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.
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- 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.
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Bacterial Mutation Test  
Preliminary Toxicity Test

Table 1

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL  
Experiment No. : A1  
Strain : TA98  
Date Plated : 7 Dec 2001  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25 µL

| Test Substance<br>Concentration<br>µg per plate | With S9 Activation      |                    | Without S9 Activation   |                    |
|-------------------------------------------------|-------------------------|--------------------|-------------------------|--------------------|
|                                                 | Revertants<br>per plate | Background<br>Lawn | Revertants<br>per plate | Background<br>Lawn |
| Vehicle                                         | 13                      | 1                  | 21                      | 1                  |
| 6.7                                             | 9                       | 1                  | 9                       | 1                  |
| 10                                              | 9                       | 1                  | 17                      | 1                  |
| 33                                              | 10                      | 1                  | 12                      | 1                  |
| 67                                              | 10                      | 1NP                | 12                      | 1NP                |
| 100                                             | 19                      | 1NP                | 16                      | 1NP                |
| 333                                             | 17                      | 1NP                | 9                       | 1NP                |
| 667                                             | 12                      | 1NP                | 8                       | 1NP                |
| 1000                                            | 10                      | 1NP                | 10                      | 1NP                |
| 3333                                            | 16                      | 1NP                | 11                      | 1NP                |
| 5000                                            | 10                      | 1NP                | 13                      | 1NP                |

Background Lawn Code

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

BioReliance

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H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

DuPont-8867

Bacterial Mutation Test  
Preliminary Toxicity Test

Table 2

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL  
Experiment No. : A1  
Strain : TA100  
Date Plated : 7 Dec 2001  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25 µL

| Test Substance<br>Concentration<br>µg per plate | With S9 Activation      |                    | Without S9 Activation   |                    |
|-------------------------------------------------|-------------------------|--------------------|-------------------------|--------------------|
|                                                 | Revertants<br>per plate | Background<br>Lawn | Revertants<br>per plate | Background<br>Lawn |
| Vehicle                                         | 148                     | 1                  | 129                     | 1                  |
| 6.7                                             | 121                     | 1                  | 152                     | 1                  |
| 10                                              | 102                     | 1                  | 114                     | 1                  |
| 33                                              | 142                     | 1                  | 139                     | 1                  |
| 67                                              | 134                     | 1NP                | 150                     | 1NP                |
| 100                                             | 147                     | 1NP                | 138                     | 1NP                |
| 333                                             | 144                     | 1NP                | 132                     | 1NP                |
| 667                                             | 134                     | 1NP                | 123                     | 1NP                |
| 1000                                            | 124                     | 1NP                | 131                     | 1NP                |
| 3333                                            | 113                     | 1NP                | 127                     | 1NP                |
| 5000                                            | 109                     | 1NP                | 142                     | 1NP                |

Background Lawn Code

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

BioReliance

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test  
Preliminary Toxicity Test**

**Table 3**

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL  
Experiment No. : A1  
Strain : TA1535  
Date Plated : 7 Dec 2001  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25 µL

| Test Substance<br>Concentration<br>µg per plate | With S9 Activation      |                    | Without S9 Activation   |                    |
|-------------------------------------------------|-------------------------|--------------------|-------------------------|--------------------|
|                                                 | Revertants<br>per plate | Background<br>Lawn | Revertants<br>per plate | Background<br>Lawn |
| Vehicle                                         | 17                      | 1                  | 12                      | 1                  |
| 6.7                                             | 9                       | 1                  | 12                      | 1                  |
| 10                                              | 7                       | 1                  | 10                      | 1                  |
| 33                                              | 11                      | 1                  | 10                      | 1                  |
| 67                                              | 18                      | 1NP                | 13                      | 1NP                |
| 100                                             | 12                      | 1NP                | 15                      | 1NP                |
| 333                                             | 11                      | 1NP                | 18                      | 1NP                |
| 667                                             | 14                      | 1NP                | 11                      | 1NP                |
| 1000                                            | 13                      | 1NP                | 13                      | 1NP                |
| 3333                                            | 13                      | 1NP                | 13                      | 1NP                |
| 5000                                            | 12                      | 1NP                | 19                      | 1NP                |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test  
Preliminary Toxicity Test**

**Table 4**

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL  
Experiment No. : A1  
Strain : TA1537  
Date Plated : 7 Dec 2001  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25 µL

| Test Substance<br>Concentration<br>µg per plate | With S9 Activation      |                    | Without S9 Activation   |                    |
|-------------------------------------------------|-------------------------|--------------------|-------------------------|--------------------|
|                                                 | Revertants<br>per plate | Background<br>Lawn | Revertants<br>per plate | Background<br>Lawn |
| Vehicle                                         | 4                       | 1                  | 5                       | 1                  |
| 6.7                                             | 5                       | 1                  | 5                       | 1                  |
| 10                                              | 7                       | 1                  | 5                       | 1                  |
| 33                                              | 5                       | 1                  | 3                       | 1                  |
| 67                                              | 8                       | 1NP                | 7                       | 1NP                |
| 100                                             | 5                       | 1NP                | 5                       | 1NP                |
| 333                                             | 2                       | 1NP                | 3                       | 1NP                |
| 667                                             | 4                       | 1NP                | 4                       | 1NP                |
| 1000                                            | 4                       | 1NP                | 5                       | 1NP                |
| 3333                                            | 8                       | 1NP                | 4                       | 1NP                |
| 5000                                            | 5                       | 1NP                | 3                       | 1NP                |

**Background Lawn Code**

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

**BioReliance**

**Study No. AA52XK.502001.BTL**

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H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

DuPont-8867

Bacterial Mutation Test  
Preliminary Toxicity Test

Table 5

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL  
Experiment No. : A1  
Strain : WP2 uvrA  
Date Plated : 7 Dec 2001  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25 µL

| Test Substance<br>Concentration<br>µg per plate | With S9 Activation      |                    | Without S9 Activation   |                    |
|-------------------------------------------------|-------------------------|--------------------|-------------------------|--------------------|
|                                                 | Revertants<br>per plate | Background<br>Lawn | Revertants<br>per plate | Background<br>Lawn |
| Vehicle                                         | 14                      | 1                  | 14                      | 1                  |
| 6.7                                             | 14                      | 1                  | 10                      | 1                  |
| 10                                              | 14                      | 1                  | 12                      | 1                  |
| 33                                              | 10                      | 1                  | 11                      | 1                  |
| 67                                              | 12                      | 1NP                | 8                       | 1                  |
| 100                                             | 13                      | 1NP                | 9                       | 1NP                |
| 333                                             | 12                      | 1NP                | 16                      | 1NP                |
| 667                                             | 10                      | 1NP                | 12                      | 1NP                |
| 1000                                            | 16                      | 1NP                | 17                      | 1NP                |
| 3333                                            | 9                       | 1NP                | 13                      | 1NP                |
| 5000                                            | 10                      | 1NP                | 10                      | 1NP                |

Background Lawn Code

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

BioReliance

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 6**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA98

Cells Seeded :  $2.4 \times 10^8$

Liver Microsomes : None

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                     | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|---------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                           | 01              | 10                      | 1                  | 10                    | 0                     |
|                                                   | 02              | 10                      | 1                  |                       |                       |
|                                                   | 03              | 10                      | 1                  |                       |                       |
| 15                                                | 01              | 14                      | 1                  | 13                    | 1                     |
|                                                   | 02              | 12                      | 1                  |                       |                       |
|                                                   | 03              | 12                      | 1                  |                       |                       |
| 50                                                | 01              | 10                      | 1                  | 10                    | 0                     |
|                                                   | 02              | 10                      | 1                  |                       |                       |
|                                                   | 03              | 10                      | 1                  |                       |                       |
| 150                                               | 01              | 12                      | 1                  | 10                    | 2                     |
|                                                   | 02              | 8                       | 1                  |                       |                       |
|                                                   | 03              | 10                      | 1                  |                       |                       |
| 500                                               | 01              | 12                      | 1NP                | 10                    | 2                     |
|                                                   | 02              | 10                      | 1NP                |                       |                       |
|                                                   | 03              | 9                       | 1NP                |                       |                       |
| 1500                                              | 01              | 10                      | 1NP                | 12                    | 3                     |
|                                                   | 02              | 10                      | 1NP                |                       |                       |
|                                                   | 03              | 16                      | 1NP                |                       |                       |
| 5000                                              | 01              | 12                      | 1NP                | 11                    | 2                     |
|                                                   | 02              | 8                       | 1NP                |                       |                       |
|                                                   | 03              | 12                      | 1NP                |                       |                       |
| Positive Control 2-nitrofluorene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                   | 01              | 157                     | 1                  | 179                   | 23                    |
|                                                   | 02              | 178                     | 1                  |                       |                       |
|                                                   | 03              | 203                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 7**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA98

Cells Seeded :  $2.4 \times 10^8$

Liver Microsomes : Rat liver S9

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 16                      | 1                  | 16                    | 7                     |
|                                                     | 02              | 10                      | 1                  |                       |                       |
|                                                     | 03              | 23                      | 1                  |                       |                       |
| 15                                                  | 01              | 17                      | 1                  | 15                    | 2                     |
|                                                     | 02              | 14                      | 1                  |                       |                       |
|                                                     | 03              | 13                      | 1                  |                       |                       |
| 50                                                  | 01              | 12                      | 1                  | 16                    | 3                     |
|                                                     | 02              | 17                      | 1                  |                       |                       |
|                                                     | 03              | 18                      | 1                  |                       |                       |
| 150                                                 | 01              | 17                      | 1                  | 18                    | 1                     |
|                                                     | 02              | 18                      | 1                  |                       |                       |
|                                                     | 03              | 19                      | 1                  |                       |                       |
| 500                                                 | 01              | 10                      | 1NP                | 15                    | 8                     |
|                                                     | 02              | 24                      | 1NP                |                       |                       |
|                                                     | 03              | 12                      | 1NP                |                       |                       |
| 1500                                                | 01              | 14                      | 1NP                | 14                    | 1                     |
|                                                     | 02              | 14                      | 1NP                |                       |                       |
|                                                     | 03              | 15                      | 1NP                |                       |                       |
| 5000                                                | 01              | 17                      | 1NP                | 16                    | 2                     |
|                                                     | 02              | 16                      | 1NP                |                       |                       |
|                                                     | 03              | 14                      | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 467                     | 1                  | 446                   | 33                    |
|                                                     | 02              | 462                     | 1                  |                       |                       |
|                                                     | 03              | 408                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 8**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA100

Cells Seeded :  $4.1 \times 10^8$

Liver Microsomes : None

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                  | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                        | 01              | 160                     | 1                  | 159                   | 5                     |
|                                                | 02              | 154                     | 1                  |                       |                       |
|                                                | 03              | 164                     | 1                  |                       |                       |
| 15                                             | 01              | 187                     | 1                  | 159                   | 24                    |
|                                                | 02              | 150                     | 1                  |                       |                       |
|                                                | 03              | 141                     | 1                  |                       |                       |
| 50                                             | 01              | 140                     | 1                  | 150                   | 11                    |
|                                                | 02              | 162                     | 1                  |                       |                       |
|                                                | 03              | 148                     | 1                  |                       |                       |
| 150                                            | 01              | 146                     | 1                  | 139                   | 10                    |
|                                                | 02              | 144                     | 1                  |                       |                       |
|                                                | 03              | 128                     | 1                  |                       |                       |
| 500                                            | 01              | 156                     | 1NP                | 143                   | 12                    |
|                                                | 02              | 133                     | 1NP                |                       |                       |
|                                                | 03              | 141                     | 1NP                |                       |                       |
| 1500                                           | 01              | 68                      | 1NP                | 124                   | 49                    |
|                                                | 02              | 151                     | 1NP                |                       |                       |
|                                                | 03              | 154                     | 1NP                |                       |                       |
| 5000                                           | 01              | 193                     | 1NP                | 166                   | 23                    |
|                                                | 02              | 153                     | 1NP                |                       |                       |
|                                                | 03              | 153                     | 1NP                |                       |                       |
| Positive Control sodium azide 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                | 01              | 491                     | 1                  | 503                   | 13                    |
|                                                | 02              | 503                     | 1                  |                       |                       |
|                                                | 03              | 516                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 9**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA100

Cells Seeded :  $4.1 \times 10^8$

Liver Microsomes : Rat liver S9

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 134                     | 1                  | 163                   | 29                    |
|                                                     | 02              | 163                     | 1                  |                       |                       |
|                                                     | 03              | 191                     | 1                  |                       |                       |
| 15                                                  | 01              | 180                     | 1                  | 171                   | 8                     |
|                                                     | 02              | 165                     | 1                  |                       |                       |
|                                                     | 03              | 167                     | 1                  |                       |                       |
| 50                                                  | 01              | 147                     | 1                  | 151                   | 12                    |
|                                                     | 02              | 141                     | 1                  |                       |                       |
|                                                     | 03              | 164                     | 1                  |                       |                       |
| 150                                                 | 01              | 235                     | 1                  | 189                   | 40                    |
|                                                     | 02              | 164                     | 1                  |                       |                       |
|                                                     | 03              | 169                     | 1                  |                       |                       |
| 500                                                 | 01              | 153                     | 1NP                | 166                   | 14                    |
|                                                     | 02              | 181                     | 1NP                |                       |                       |
|                                                     | 03              | 163                     | 1NP                |                       |                       |
| 1500                                                | 01              | 193                     | 1NP                | 176                   | 25                    |
|                                                     | 02              | 187                     | 1NP                |                       |                       |
|                                                     | 03              | 147                     | 1NP                |                       |                       |
| 5000                                                | 01              | 196                     | 1NP                | 177                   | 16                    |
|                                                     | 02              | 171                     | 1NP                |                       |                       |
|                                                     | 03              | 165                     | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 763                     | 1                  | 657                   | 128                   |
|                                                     | 02              | 692                     | 1                  |                       |                       |
|                                                     | 03              | 515                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 10**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA1535

Cells Seeded :  $2.7 \times 10^8$

Liver Microsomes : None

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                  | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                        | 01              | 12                      | 1                  | 13                    | 2                     |
|                                                | 02              | 15                      | 1                  |                       |                       |
|                                                | 03              | 13                      | 1                  |                       |                       |
| 15                                             | 01              | 18                      | 1                  | 14                    | 4                     |
|                                                | 02              | 14                      | 1                  |                       |                       |
|                                                | 03              | 10                      | 1                  |                       |                       |
| 50                                             | 01              | 10                      | 1                  | 11                    | 1                     |
|                                                | 02              | 12                      | 1                  |                       |                       |
|                                                | 03              | 12                      | 1                  |                       |                       |
| 150                                            | 01              | 11                      | 1                  | 12                    | 2                     |
|                                                | 02              | 15                      | 1                  |                       |                       |
|                                                | 03              | 11                      | 1                  |                       |                       |
| 500                                            | 01              | 10                      | 1NP                | 9                     | 1                     |
|                                                | 02              | 10                      | 1NP                |                       |                       |
|                                                | 03              | 8                       | 1NP                |                       |                       |
| 1500                                           | 01              | 10                      | 1NP                | 11                    | 1                     |
|                                                | 02              | 12                      | 1NP                |                       |                       |
|                                                | 03              | 12                      | 1NP                |                       |                       |
| 5000                                           | 01              | 12                      | 1NP                | 13                    | 1                     |
|                                                | 02              | 13                      | 1NP                |                       |                       |
|                                                | 03              | 13                      | 1NP                |                       |                       |
| Positive Control sodium azide 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                | 01              | 297                     | 1                  | 280                   | 44                    |
|                                                | 02              | 312                     | 1                  |                       |                       |
|                                                | 03              | 230                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 11**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA1535

Cells Seeded :  $2.7 \times 10^8$

Liver Microsomes : Rat liver S9

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 9                       | 1                  | 10                    | 2                     |
|                                                     | 02              | 9                       | 1                  |                       |                       |
|                                                     | 03              | 13                      | 1                  |                       |                       |
| 15                                                  | 01              | 12                      | 1                  | 10                    | 2                     |
|                                                     | 02              | 9                       | 1                  |                       |                       |
|                                                     | 03              | 10                      | 1                  |                       |                       |
| 50                                                  | 01              | 9                       | 1                  | 12                    | 3                     |
|                                                     | 02              | 14                      | 1                  |                       |                       |
|                                                     | 03              | 12                      | 1                  |                       |                       |
| 150                                                 | 01              | 12                      | 1                  | 8                     | 4                     |
|                                                     | 02              | 5                       | 1                  |                       |                       |
|                                                     | 03              | 8                       | 1                  |                       |                       |
| 500                                                 | 01              | 20                      | 1NP                | 16                    | 3                     |
|                                                     | 02              | 14                      | 1NP                |                       |                       |
|                                                     | 03              | 15                      | 1NP                |                       |                       |
| 1500                                                | 01              | 11                      | 1NP                | 9                     | 3                     |
|                                                     | 02              | 9                       | 1NP                |                       |                       |
|                                                     | 03              | 6                       | 1NP                |                       |                       |
| 5000                                                | 01              | 14                      | 1NP                | 10                    | 5                     |
|                                                     | 02              | 5                       | 1NP                |                       |                       |
|                                                     | 03              | 12                      | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 121                     | 1                  | 111                   | 23                    |
|                                                     | 02              | 85                      | 1                  |                       |                       |
|                                                     | 03              | 128                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 12**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA1537

Cells Seeded :  $1.6 \times 10^8$

Liver Microsomes : None

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                    | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|--------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                          | 01              | 5                       | 1                  | 4                     | 2                     |
|                                                  | 02              | 2                       | 1                  |                       |                       |
|                                                  | 03              | 4                       | 1                  |                       |                       |
| 15                                               | 01              | 2                       | 1                  | 4                     | 2                     |
|                                                  | 02              | 6                       | 1                  |                       |                       |
|                                                  | 03              | 4                       | 1                  |                       |                       |
| 50                                               | 01              | 8                       | 1                  | 5                     | 3                     |
|                                                  | 02              | 3                       | 1                  |                       |                       |
|                                                  | 03              | 4                       | 1                  |                       |                       |
| 150                                              | 01              | 6                       | 1                  | 4                     | 2                     |
|                                                  | 02              | 3                       | 1                  |                       |                       |
|                                                  | 03              | 3                       | 1                  |                       |                       |
| 500                                              | 01              | 4                       | 1NP                | 5                     | 1                     |
|                                                  | 02              | 5                       | 1NP                |                       |                       |
|                                                  | 03              | 6                       | 1NP                |                       |                       |
| 1500                                             | 01              | 8                       | 1NP                | 6                     | 2                     |
|                                                  | 02              | 7                       | 1NP                |                       |                       |
|                                                  | 03              | 4                       | 1NP                |                       |                       |
| 5000                                             | 01              | 6                       | 1NP                | 5                     | 2                     |
|                                                  | 02              | 3                       | 1NP                |                       |                       |
|                                                  | 03              | 5                       | 1NP                |                       |                       |
| Positive Control 9-aminoacridine 75 µg per plate |                 |                         |                    |                       |                       |
|                                                  | 01              | 605                     | 1                  | 692                   | 126                   |
|                                                  | 02              | 837                     | 1                  |                       |                       |
|                                                  | 03              | 635                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 13**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : TA1537

Cells Seeded :  $1.6 \times 10^8$

Liver Microsomes : Rat liver S9

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 5                       | 1                  | 6                     | 3                     |
|                                                     | 02              | 3                       | 1                  |                       |                       |
|                                                     | 03              | 9                       | 1                  |                       |                       |
| 15                                                  | 01              | 12                      | 1                  | 8                     | 3                     |
|                                                     | 02              | 6                       | 1                  |                       |                       |
|                                                     | 03              | 7                       | 1                  |                       |                       |
| 50                                                  | 01              | 4                       | 1                  | 5                     | 1                     |
|                                                     | 02              | 5                       | 1                  |                       |                       |
|                                                     | 03              | 5                       | 1                  |                       |                       |
| 150                                                 | 01              | 10                      | 1                  | 7                     | 3                     |
|                                                     | 02              | 5                       | 1                  |                       |                       |
|                                                     | 03              | 5                       | 1                  |                       |                       |
| 500                                                 | 01              | 9                       | 1NP                | 6                     | 2                     |
|                                                     | 02              | 5                       | 1NP                |                       |                       |
|                                                     | 03              | 5                       | 1NP                |                       |                       |
| 1500                                                | 01              | 5                       | 1NP                | 6                     | 1                     |
|                                                     | 02              | 5                       | 1NP                |                       |                       |
|                                                     | 03              | 7                       | 1NP                |                       |                       |
| 5000                                                | 01              | 5                       | 1NP                | 6                     | 2                     |
|                                                     | 02              | 8                       | 1NP                |                       |                       |
|                                                     | 03              | 6                       | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 101                     | 1                  | 94                    | 8                     |
|                                                     | 02              | 96                      | 1                  |                       |                       |
|                                                     | 03              | 86                      | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 14**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Strain : WP2 uvrA

Cells Seeded :  $11.2 \times 10^8$

Liver Microsomes : None

Date Plated : 19 Dec 2001

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                              | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|------------------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                                    | 01              | 15                      | 1                  | 13                    | 3                     |
|                                                            | 02              | 10                      | 1                  |                       |                       |
|                                                            | 03              | 13                      | 1                  |                       |                       |
| 15                                                         | 01              | 10                      | 1                  | 10                    | 0                     |
|                                                            | 02              | 10                      | 1                  |                       |                       |
|                                                            | 03              | 10                      | 1                  |                       |                       |
| 50                                                         | 01              | 12                      | 1                  | 12                    | 0                     |
|                                                            | 02              | 12                      | 1                  |                       |                       |
|                                                            | 03              | 12                      | 1                  |                       |                       |
| 150                                                        | 01              | 14                      | 1                  | 13                    | 1                     |
|                                                            | 02              | 14                      | 1                  |                       |                       |
|                                                            | 03              | 12                      | 1                  |                       |                       |
| 500                                                        | 01              | 12                      | 1NP                | 12                    | 0                     |
|                                                            | 02              | 12                      | 1NP                |                       |                       |
|                                                            | 03              | 12                      | 1NP                |                       |                       |
| 1500                                                       | 01              | 13                      | 1NP                | 12                    | 2                     |
|                                                            | 02              | 10                      | 1NP                |                       |                       |
|                                                            | 03              | 13                      | 1NP                |                       |                       |
| 5000                                                       | 01              | 17                      | 1NP                | 14                    | 3                     |
|                                                            | 02              | 11                      | 1NP                |                       |                       |
|                                                            | 03              | 13                      | 1NP                |                       |                       |
| Positive Control methyl methanesulfonate 1000 µg per plate |                 |                         |                    |                       |                       |
|                                                            | 01              | 113                     | 1                  | 95                    | 18                    |
|                                                            | 02              | 96                      | 1                  |                       |                       |
|                                                            | 03              | 77                      | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 15**

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL Experiment No : B1  
Strain : WP2 *uvrA* Cells Seeded :  $11.2 \times 10^8$   
Liver Microsomes : Rat liver S9 Date Plated : 19 Dec 2001  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                      | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                            | 01              | 10                      | 1                  | 12                    | 3                     |
|                                                    | 02              | 10                      | 1                  |                       |                       |
|                                                    | 03              | 16                      | 1                  |                       |                       |
| 15                                                 | 01              | 11                      | 1                  | 11                    | 1                     |
|                                                    | 02              | 12                      | 1                  |                       |                       |
|                                                    | 03              | 10                      | 1                  |                       |                       |
| 50                                                 | 01              | 11                      | 1                  | 10                    | 1                     |
|                                                    | 02              | 10                      | 1                  |                       |                       |
|                                                    | 03              | 10                      | 1                  |                       |                       |
| 150                                                | 01              | 10                      | 1                  | 10                    | 0                     |
|                                                    | 02              | 10                      | 1                  |                       |                       |
|                                                    | 03              | 10                      | 1                  |                       |                       |
| 500                                                | 01              | 11                      | 1NP                | 13                    | 2                     |
|                                                    | 02              | 14                      | 1NP                |                       |                       |
|                                                    | 03              | 13                      | 1NP                |                       |                       |
| 1500                                               | 01              | 5                       | 1NP                | 9                     | 4                     |
|                                                    | 02              | 10                      | 1NP                |                       |                       |
|                                                    | 03              | 13                      | 1NP                |                       |                       |
| 5000                                               | 01              | 17                      | 1NP                | 15                    | 2                     |
|                                                    | 02              | 14                      | 1NP                |                       |                       |
|                                                    | 03              | 14                      | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 10 µg per plate |                 |                         |                    |                       |                       |
|                                                    | 01              | 713                     | 1                  | 515                   | 171                   |
|                                                    | 02              | 413                     | 1                  |                       |                       |
|                                                    | 03              | 420                     | 1                  |                       |                       |

**Background Lawn Code**

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

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 16**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Strain : TA98

Cells Seeded :  $2.7 \times 10^8$

Liver Microsomes : None

Date Plated : 4 Jan 2002

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                     | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|---------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                           | 01              | 22                      | 1                  | 19                    | 4                     |
|                                                   | 02              | 19                      | 1                  |                       |                       |
|                                                   | 03              | 15                      | 1                  |                       |                       |
| 15                                                | 01              | 9                       | 1                  | 16                    | 6                     |
|                                                   | 02              | 18                      | 1                  |                       |                       |
|                                                   | 03              | 20                      | 1                  |                       |                       |
| 50                                                | 01              | 11                      | 1                  | 12                    | 5                     |
|                                                   | 02              | 17                      | 1                  |                       |                       |
|                                                   | 03              | 8                       | 1                  |                       |                       |
| 150                                               | 01              | 9                       | 1                  | 10                    | 1                     |
|                                                   | 02              | 10                      | 1                  |                       |                       |
|                                                   | 03              | 10                      | 1                  |                       |                       |
| 500                                               | 01              | 10                      | 1IP                | 12                    | 5                     |
|                                                   | 02              | 17                      | 1NP                |                       |                       |
|                                                   | 03              | 8                       | 1IP                |                       |                       |
| 1500                                              | 01              | 8                       | 1NP                | 5                     | 3                     |
|                                                   | 02              | 2                       | 1NP                |                       |                       |
|                                                   | 03              | 5                       | 1NP                |                       |                       |
| 5000                                              | 01              | 11                      | 1IP                | 9                     | 3                     |
|                                                   | 02              | 6                       | 1IP                |                       |                       |
|                                                   | 03              | 9                       | 1IP                |                       |                       |
| Positive Control 2-nitrofluorene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                   | 01              | 247                     | 1                  | 238                   | 10                    |
|                                                   | 02              | 239                     | 1                  |                       |                       |
|                                                   | 03              | 228                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

DuPont-8867

Bacterial Mutation Test

Table 17

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Strain : TA98

Cells Seeded :  $2.7 \times 10^8$

Liver Microsomes : Rat liver S9

Date Plated : 4 Jan 2002

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 22                      | 1                  | 24                    | 2                     |
|                                                     | 02              | 24                      | 1                  |                       |                       |
|                                                     | 03              | 25                      | 1                  |                       |                       |
| 15                                                  | 01              | 28                      | 1                  | 19                    | 9                     |
|                                                     | 02              | 11                      | 1                  |                       |                       |
|                                                     | 03              | 18                      | 1                  |                       |                       |
| 50                                                  | 01              | 23                      | 1                  | 23                    | 3                     |
|                                                     | 02              | 20                      | 1                  |                       |                       |
|                                                     | 03              | 26                      | 1                  |                       |                       |
| 150                                                 | 01              | 15                      | 1                  | 17                    | 3                     |
|                                                     | 02              | 20                      | 1                  |                       |                       |
|                                                     | 03              | 15                      | 1                  |                       |                       |
| 500                                                 | 01              | 17                      | 1NP                | 20                    | 6                     |
|                                                     | 02              | 16                      | 1NP                |                       |                       |
|                                                     | 03              | 26                      | 1NP                |                       |                       |
| 1500                                                | 01              | 29                      | 1IP                | 23                    | 7                     |
|                                                     | 02              | 16                      | 1IP                |                       |                       |
|                                                     | 03              | 25                      | 1IP                |                       |                       |
| 5000                                                | 01              | 17                      | 1IP                | 19                    | 4                     |
|                                                     | 02              | 16                      | 1NP                |                       |                       |
|                                                     | 03              | 24                      | 1IP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 1011                    | 1                  | 1011                  | 36                    |
|                                                     | 02              | 975                     | 1                  |                       |                       |
|                                                     | 03              | 1046                    | 1                  |                       |                       |

Background Lawn Code

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

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NP=Non-Interfering precipitate; IP=Interfering precipitate

BioReliance

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 18**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Strain : TA100

Cells Seeded :  $1.1 \times 10^8$

Liver Microsomes : None

Date Plated : 4 Jan 2002

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                  | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                        | 01              | 191                     | 1                  | 194                   | 22                    |
|                                                | 02              | 217                     | 1                  |                       |                       |
|                                                | 03              | 174                     | 1                  |                       |                       |
| 15                                             | 01              | 182                     | 1                  | 182                   | 7                     |
|                                                | 02              | 188                     | 1                  |                       |                       |
|                                                | 03              | 175                     | 1                  |                       |                       |
| 50                                             | 01              | 186                     | 1                  | 190                   | 9                     |
|                                                | 02              | 184                     | 1                  |                       |                       |
|                                                | 03              | 200                     | 1                  |                       |                       |
| 150                                            | 01              | 194                     | 1                  | 184                   | 21                    |
|                                                | 02              | 160                     | 1                  |                       |                       |
|                                                | 03              | 197                     | 1                  |                       |                       |
| 500                                            | 01              | 221                     | 1NP                | 184                   | 32                    |
|                                                | 02              | 169                     | 1NP                |                       |                       |
|                                                | 03              | 163                     | 1NP                |                       |                       |
| 1500                                           | 01              | 178                     | 1NP                | 190                   | 22                    |
|                                                | 02              | 215                     | 1NP                |                       |                       |
|                                                | 03              | 176                     | 1NP                |                       |                       |
| 5000                                           | 01              | 181                     | 1NP                | 186                   | 8                     |
|                                                | 02              | 183                     | 1NP                |                       |                       |
|                                                | 03              | 195                     | 1NP                |                       |                       |
| Positive Control sodium azide 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                | 01              | 764                     | 1                  | 771                   | 6                     |
|                                                | 02              | 775                     | 1                  |                       |                       |
|                                                | 03              | 775                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL

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H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

DuPont-8867

Bacterial Mutation Test

Table 19

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Strain : TA100

Cells Seeded :  $1.1 \times 10^8$

Liver Microsomes : Rat liver S9

Date Plated : 4 Jan 2002

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 163                     | 1                  | 188                   | 21                    |
|                                                     | 02              | 201                     | 1                  |                       |                       |
|                                                     | 03              | 199                     | 1                  |                       |                       |
| 15                                                  | 01              | 204                     | 1                  | 191                   | 12                    |
|                                                     | 02              | 185                     | 1                  |                       |                       |
|                                                     | 03              | 183                     | 1                  |                       |                       |
| 50                                                  | 01              | 176                     | 1                  | 189                   | 17                    |
|                                                     | 02              | 182                     | 1                  |                       |                       |
|                                                     | 03              | 208                     | 1                  |                       |                       |
| 150                                                 | 01              | 151                     | 1                  | 159                   | 7                     |
|                                                     | 02              | 162                     | 1                  |                       |                       |
|                                                     | 03              | 165                     | 1                  |                       |                       |
| 500                                                 | 01              | 179                     | 1NP                | 189                   | 29                    |
|                                                     | 02              | 221                     | 1NP                |                       |                       |
|                                                     | 03              | 166                     | 1                  |                       |                       |
| 1500                                                | 01              | 197                     | 1NP                | 185                   | 17                    |
|                                                     | 02              | 193                     | 1NP                |                       |                       |
|                                                     | 03              | 166                     | 1NP                |                       |                       |
| 5000                                                | 01              | 192                     | 1NP                | 195                   | 8                     |
|                                                     | 02              | 189                     | 1NP                |                       |                       |
|                                                     | 03              | 204                     | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 907                     | 1                  | 998                   | 89                    |
|                                                     | 02              | 1002                    | 1                  |                       |                       |
|                                                     | 03              | 1084                    | 1                  |                       |                       |

Background Lawn Code

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

BioReliance

Study No. AA52XK.502001.BTL

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H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

DuPont-8867

Bacterial Mutation Test

Table 20

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Strain : TA1535

Cells Seeded :  $2.6 \times 10^8$

Liver Microsomes : None

Date Plated : 4 Jan 2002

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                  | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                        | 01              | 18                      | 1                  | 18                    | 0                     |
|                                                | 02              | 18                      | 1                  |                       |                       |
|                                                | 03              | 18                      | 1                  |                       |                       |
| 15                                             | 01              | 10                      | 1                  | 16                    | 6                     |
|                                                | 02              | 20                      | 1                  |                       |                       |
|                                                | 03              | 19                      | 1                  |                       |                       |
| 50                                             | 01              | 23                      | 1                  | 20                    | 4                     |
|                                                | 02              | 21                      | 1                  |                       |                       |
|                                                | 03              | 15                      | 1                  |                       |                       |
| 150                                            | 01              | 12                      | 1                  | 15                    | 4                     |
|                                                | 02              | 13                      | 1                  |                       |                       |
|                                                | 03              | 19                      | 1                  |                       |                       |
| 500                                            | 01              | 20                      | 1NP                | 23                    | 4                     |
|                                                | 02              | 21                      | 1NP                |                       |                       |
|                                                | 03              | 28                      | 1                  |                       |                       |
| 1500                                           | 01              | 17                      | 1NP                | 16                    | 3                     |
|                                                | 02              | 13                      | 1IP                |                       |                       |
|                                                | 03              | 18                      | 1NP                |                       |                       |
| 5000                                           | 01              | 16                      | 1IP                | 16                    | 2                     |
|                                                | 02              | 14                      | 1IP                |                       |                       |
|                                                | 03              | 18                      | 1IP                |                       |                       |
| Positive Control sodium azide 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                | 01              | 420                     | 1                  | 437                   | 41                    |
|                                                | 02              | 484                     | 1                  |                       |                       |
|                                                | 03              | 408                     | 1                  |                       |                       |

Background Lawn Code

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

BioReliance

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 21**

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL Experiment No : B2  
Strain : TA1535 Cells Seeded :  $2.6 \times 10^8$   
Liver Microsomes : Rat liver S9 Date Plated : 4 Jan 2002  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 12                      | 1                  | 15                    | 3                     |
|                                                     | 02              | 14                      | 1                  |                       |                       |
|                                                     | 03              | 18                      | 1                  |                       |                       |
| 15                                                  | 01              | 19                      | 1                  | 17                    | 3                     |
|                                                     | 02              | 14                      | 1                  |                       |                       |
|                                                     | 03              | 17                      | 1                  |                       |                       |
| 50                                                  | 01              | 20                      | 1                  | 16                    | 5                     |
|                                                     | 02              | 16                      | 1                  |                       |                       |
|                                                     | 03              | 11                      | 1                  |                       |                       |
| 150                                                 | 01              | 13                      | 1                  | 12                    | 2                     |
|                                                     | 02              | 14                      | 1                  |                       |                       |
|                                                     | 03              | 10                      | 1                  |                       |                       |
| 500                                                 | 01              | 8                       | 1NP                | 14                    | 6                     |
|                                                     | 02              | 20                      | 1NP                |                       |                       |
|                                                     | 03              | 14                      | 1NP                |                       |                       |
| 1500                                                | 01              | 15                      | 1NP                | 16                    | 2                     |
|                                                     | 02              | 18                      | 1IP                |                       |                       |
|                                                     | 03              | 15                      | 1NP                |                       |                       |
| 5000                                                | 01              | 13                      | 1IP                | 10                    | 4                     |
|                                                     | 02              | 6                       | 1IP                |                       |                       |
|                                                     | 03              | 11                      | 1IP                |                       |                       |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 168                     | 1                  | 166                   | 8                     |
|                                                     | 02              | 173                     | 1                  |                       |                       |
|                                                     | 03              | 158                     | 1                  |                       |                       |

**Background Lawn Code**

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

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 22**

Test Substance Id: H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Strain : TA1537

Cells Seeded :  $1.3 \times 10^8$

Liver Microsomes : None

Date Plated : 4 Jan 2002

Vehicle : tetrahydrofuran

Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                    | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|--------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                          | 01              | 8                       | 1                  | 6                     | 2                     |
|                                                  | 02              | 6                       | 1                  |                       |                       |
|                                                  | 03              | 5                       | 1                  |                       |                       |
| 15                                               | 01              | 7                       | 1                  | 6                     | 1                     |
|                                                  | 02              | 5                       | 1                  |                       |                       |
|                                                  | 03              | 5                       | 1                  |                       |                       |
| 50                                               | 01              | 10                      | 1                  | 7                     | 4                     |
|                                                  | 02              | 3                       | 1                  |                       |                       |
|                                                  | 03              | 8                       | 1                  |                       |                       |
| 150                                              | 01              | 10                      | 1                  | 6                     | 3                     |
|                                                  | 02              | 5                       | 1                  |                       |                       |
|                                                  | 03              | 4                       | 1                  |                       |                       |
| 500                                              | 01              | 6                       | 1IP                | 5                     | 2                     |
|                                                  | 02              | 7                       | 1IP                |                       |                       |
|                                                  | 03              | 3                       | 1NP                |                       |                       |
| 1500                                             | 01              | 9                       | 1NP                | 6                     | 3                     |
|                                                  | 02              | 4                       | 1IP                |                       |                       |
|                                                  | 03              | 4                       | 1IP                |                       |                       |
| 5000                                             | 01              | 3                       | 1NP                | 3                     | 1                     |
|                                                  | 02              | 3                       | 1IP                |                       |                       |
|                                                  | 03              | 2                       | 1NP                |                       |                       |
| Positive Control 9-aminoacridine 75 µg per plate |                 |                         |                    |                       |                       |
|                                                  | 01              | 696                     | 1                  | 760                   | 173                   |
|                                                  | 02              | 629                     | 1                  |                       |                       |
|                                                  | 03              | 956                     | 1                  |                       |                       |

**Background Lawn Code**

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

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

NP=Non-Interfering precipitate; IP=Interfering precipitate

**BioReliance**

Study No. AA52XK.502001.BTL



**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 23**

Test Substance Id: H-25170  
 Study Number : AA52XK.502001.BTL Experiment No : B2  
 Strain : TA1537 Cells Seeded :  $1.3 \times 10^8$   
 Liver Microsomes : Rat liver S9 Date Plated : 4 Jan 2002  
 Vehicle : tetrahydrofuran  
 Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                       | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|-----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                             | 01              | 8                       | 1                  |                       |                       |
|                                                     | 02              | 9                       | 1                  |                       |                       |
|                                                     | 03              | 10                      | 1                  | 9                     | 1                     |
| 15                                                  | 01              | 12                      | 1                  |                       |                       |
|                                                     | 02              | 4                       | 1                  |                       |                       |
|                                                     | 03              | 10                      | 1                  | 9                     | 4                     |
| 50                                                  | 01              | 8                       | 1                  |                       |                       |
|                                                     | 02              | 4                       | 1                  |                       |                       |
|                                                     | 03              | 1                       | 1                  | 4                     | 4                     |
| 150                                                 | 01              | 5                       | 1                  |                       |                       |
|                                                     | 02              | 10                      | 1                  |                       |                       |
|                                                     | 03              | 11                      | 1                  | 9                     | 3                     |
| 500                                                 | 01              | 3                       | 1NP                |                       |                       |
|                                                     | 02              | 2                       | 1NP                |                       |                       |
|                                                     | 03              | 9                       | 1NP                | 5                     | 4                     |
| 1500                                                | 01              | 8                       | 1NP                |                       |                       |
|                                                     | 02              | 7                       | 1IP                |                       |                       |
|                                                     | 03              | 9                       | 1IP                | 8                     | 1                     |
| 5000                                                | 01              | 9                       | 1IP                |                       |                       |
|                                                     | 02              | 6                       | 1NP                |                       |                       |
|                                                     | 03              | 3                       | 1NP                | 6                     | 3                     |
| Positive Control 2-aminoanthracene 1.0 µg per plate |                 |                         |                    |                       |                       |
|                                                     | 01              | 216                     | 1                  |                       |                       |
|                                                     | 02              | 113                     | 1                  |                       |                       |
|                                                     | 03              | 178                     | 1                  | 169                   | 52                    |

**Background Lawn Code**

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

**BioReliance**

Study No. AA52XK.502001.BTL

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 24**

Test Substance Id: H-25170  
Study Number : AA52XK.502001.BTL Experiment No : B2  
Strain : WP2 uvrA Cells Seeded : 2.5 X 10<sup>8</sup>  
Liver Microsomes : None Date Plated : 4 Jan 2002  
Vehicle : tetrahydrofuran  
Plating Aliquot : 25 µL

| Concentration<br>µg per plate                              | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|------------------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                                    | 01              | 10                      | 1                  | 10                    | 2                     |
|                                                            | 02              | 9                       | 1                  |                       |                       |
|                                                            | 03              | 12                      | 1                  |                       |                       |
| 15                                                         | 01              | 14                      | 1                  | 14                    | 1                     |
|                                                            | 02              | 14                      | 1                  |                       |                       |
|                                                            | 03              | 13                      | 1                  |                       |                       |
| 50                                                         | 01              | 12                      | 1                  | 12                    | 4                     |
|                                                            | 02              | 9                       | 1                  |                       |                       |
|                                                            | 03              | 16                      | 1                  |                       |                       |
| 150                                                        | 01              | 21                      | 1                  | 14                    | 8                     |
|                                                            | 02              | 14                      | 1                  |                       |                       |
|                                                            | 03              | 6                       | 1                  |                       |                       |
| 500                                                        | 01              | 10                      | 1                  | 13                    | 4                     |
|                                                            | 02              | 12                      | 1                  |                       |                       |
|                                                            | 03              | 17                      | 1                  |                       |                       |
| 1500                                                       | 01              | 5                       | 1NP                | 10                    | 4                     |
|                                                            | 02              | 12                      | 1NP                |                       |                       |
|                                                            | 03              | 13                      | 1NP                |                       |                       |
| 5000                                                       | 01              | 9                       | 1NP                | 10                    | 2                     |
|                                                            | 02              | 12                      | 1NP                |                       |                       |
|                                                            | 03              | 8                       | 1NP                |                       |                       |
| Positive Control methyl methanesulfonate 1000 µg per plate |                 |                         |                    |                       |                       |
|                                                            | 01              | 108                     | 1                  | 117                   | 8                     |
|                                                            | 02              | 121                     | 1                  |                       |                       |
|                                                            | 03              | 122                     | 1                  |                       |                       |

**Background Lawn Code**

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

**BioReliance**

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test**

**Table 25**

Test Substance Id: H-25170  
 Study Number : AA52XK.502001.BTL Experiment No : B2  
 Strain : WP2 uvrA Cells Seeded :  $2.5 \times 10^8$   
 Liver Microsomes : Rat liver S9 Date Plated : 4 Jan 2002  
 Vehicle : tetrahydrofuran  
 Plating Aliquot : 25  $\mu$ L

| Concentration<br>µg per plate                      | Plate<br>Number | Revertants<br>per plate | Background<br>Code | Average<br>Revertants | Standard<br>Deviation |
|----------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------|-----------------------|
| Vehicle                                            | 01              | 9                       | 1                  | 10                    | 2                     |
|                                                    | 02              | 9                       | 1                  |                       |                       |
|                                                    | 03              | 12                      | 1                  |                       |                       |
| 15                                                 | 01              | 10                      | 1                  | 7                     | 3                     |
|                                                    | 02              | 5                       | 1                  |                       |                       |
|                                                    | 03              | 7                       | 1                  |                       |                       |
| 50                                                 | 01              | 9                       | 1                  | 11                    | 2                     |
|                                                    | 02              | 11                      | 1                  |                       |                       |
|                                                    | 03              | 13                      | 1                  |                       |                       |
| 150                                                | 01              | 10                      | 1                  | 10                    | 0                     |
|                                                    | 02              | 10                      | 1                  |                       |                       |
|                                                    | 03              | 10                      | 1                  |                       |                       |
| 500                                                | 01              | 14                      | 1                  | 11                    | 3                     |
|                                                    | 02              | 8                       | 1                  |                       |                       |
|                                                    | 03              | 11                      | 1                  |                       |                       |
| 1500                                               | 01              | 14                      | 1NP                | 14                    | 3                     |
|                                                    | 02              | 12                      | 1NP                |                       |                       |
|                                                    | 03              | 17                      | 1                  |                       |                       |
| 5000                                               | 01              | 11                      | 1NP                | 12                    | 4                     |
|                                                    | 02              | 16                      | 1NP                |                       |                       |
|                                                    | 03              | 9                       | 1NP                |                       |                       |
| Positive Control 2-aminoanthracene 10 µg per plate |                 |                         |                    |                       |                       |
|                                                    | 01              | 972                     | 1                  | 993                   | 161                   |
|                                                    | 02              | 1164                    | 1                  |                       |                       |
|                                                    | 03              | 844                     | 1                  |                       |                       |

**Background Lawn Code**

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

**BioReliance**

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**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

**Bacterial Mutation Test  
Summary of Results**

**Table 26**

Test Substance Id : H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B1

Average Revertants Per Plate  $\pm$  Standard Deviation

Liver Microsomes: None

| Dose ( $\mu$ g/plate) | TA98         | TA100        | TA1535       | TA1537        | WP2 uvrA    |
|-----------------------|--------------|--------------|--------------|---------------|-------------|
| Vehicle               | 10 $\pm$ 0   | 159 $\pm$ 5  | 13 $\pm$ 2   | 4 $\pm$ 2     | 13 $\pm$ 3  |
| 15                    | 13 $\pm$ 1   | 159 $\pm$ 24 | 14 $\pm$ 4   | 4 $\pm$ 2     | 10 $\pm$ 0  |
| 50                    | 10 $\pm$ 0   | 150 $\pm$ 11 | 11 $\pm$ 1   | 5 $\pm$ 3     | 12 $\pm$ 0  |
| 150                   | 10 $\pm$ 2   | 139 $\pm$ 10 | 12 $\pm$ 2   | 4 $\pm$ 2     | 13 $\pm$ 1  |
| 500                   | 10 $\pm$ 2   | 143 $\pm$ 12 | 9 $\pm$ 1    | 5 $\pm$ 1     | 12 $\pm$ 0  |
| 1500                  | 12 $\pm$ 3   | 124 $\pm$ 49 | 11 $\pm$ 1   | 6 $\pm$ 2     | 12 $\pm$ 2  |
| 5000                  | 11 $\pm$ 2   | 166 $\pm$ 23 | 13 $\pm$ 1   | 5 $\pm$ 2     | 14 $\pm$ 3  |
| Positive              | 179 $\pm$ 23 | 503 $\pm$ 13 | 280 $\pm$ 44 | 692 $\pm$ 126 | 95 $\pm$ 18 |

Liver Microsomes: Rat liver S9

| Dose ( $\mu$ g/plate) | TA98         | TA100         | TA1535       | TA1537     | WP2 uvrA      |
|-----------------------|--------------|---------------|--------------|------------|---------------|
| Vehicle               | 16 $\pm$ 7   | 163 $\pm$ 29  | 10 $\pm$ 2   | 6 $\pm$ 3  | 12 $\pm$ 3    |
| 15                    | 15 $\pm$ 2   | 171 $\pm$ 8   | 10 $\pm$ 2   | 8 $\pm$ 3  | 11 $\pm$ 1    |
| 50                    | 16 $\pm$ 3   | 151 $\pm$ 12  | 12 $\pm$ 3   | 5 $\pm$ 1  | 10 $\pm$ 1    |
| 150                   | 18 $\pm$ 1   | 189 $\pm$ 40  | 8 $\pm$ 4    | 7 $\pm$ 3  | 10 $\pm$ 0    |
| 500                   | 15 $\pm$ 8   | 166 $\pm$ 14  | 16 $\pm$ 3   | 6 $\pm$ 2  | 13 $\pm$ 2    |
| 1500                  | 14 $\pm$ 1   | 176 $\pm$ 25  | 9 $\pm$ 3    | 6 $\pm$ 1  | 9 $\pm$ 4     |
| 5000                  | 16 $\pm$ 2   | 177 $\pm$ 16  | 10 $\pm$ 5   | 6 $\pm$ 2  | 15 $\pm$ 2    |
| Positive              | 446 $\pm$ 33 | 657 $\pm$ 128 | 111 $\pm$ 23 | 94 $\pm$ 8 | 515 $\pm$ 171 |

Vehicle = Vehicle Control

Positive = Positive Control

Plating aliquot: 25  $\mu$ L

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H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

DuPont-8867

Bacterial Mutation Test  
Summary of Results

Table 27

Test Substance Id : H-25170

Study Number : AA52XK.502001.BTL

Experiment No : B2

Average Revertants Per Plate  $\pm$  Standard Deviation

Liver Microsomes: None

| Dose ( $\mu$ g/plate) | TA98         | TA100        | TA1535       | TA1537        | WP2 uvrA    |
|-----------------------|--------------|--------------|--------------|---------------|-------------|
| Vehicle               | 19 $\pm$ 4   | 194 $\pm$ 22 | 18 $\pm$ 0   | 6 $\pm$ 2     | 10 $\pm$ 2  |
| 15                    | 16 $\pm$ 6   | 182 $\pm$ 7  | 16 $\pm$ 6   | 6 $\pm$ 1     | 14 $\pm$ 1  |
| 50                    | 12 $\pm$ 5   | 190 $\pm$ 9  | 20 $\pm$ 4   | 7 $\pm$ 4     | 12 $\pm$ 4  |
| 150                   | 10 $\pm$ 1   | 184 $\pm$ 21 | 15 $\pm$ 4   | 6 $\pm$ 3     | 14 $\pm$ 8  |
| 500                   | 12 $\pm$ 5   | 184 $\pm$ 32 | 23 $\pm$ 4   | 5 $\pm$ 2     | 13 $\pm$ 4  |
| 1500                  | 5 $\pm$ 3    | 190 $\pm$ 22 | 16 $\pm$ 3   | 6 $\pm$ 3     | 10 $\pm$ 4  |
| 5000                  | 9 $\pm$ 3    | 186 $\pm$ 8  | 16 $\pm$ 2   | 3 $\pm$ 1     | 10 $\pm$ 2  |
| Positive              | 238 $\pm$ 10 | 771 $\pm$ 6  | 437 $\pm$ 41 | 760 $\pm$ 173 | 117 $\pm$ 8 |

Liver Microsomes: Rat liver S9

| Dose ( $\mu$ g/plate) | TA98          | TA100        | TA1535      | TA1537       | WP2 uvrA      |
|-----------------------|---------------|--------------|-------------|--------------|---------------|
| Vehicle               | 24 $\pm$ 2    | 188 $\pm$ 21 | 15 $\pm$ 3  | 9 $\pm$ 1    | 10 $\pm$ 2    |
| 15                    | 19 $\pm$ 9    | 191 $\pm$ 12 | 17 $\pm$ 3  | 9 $\pm$ 4    | 7 $\pm$ 3     |
| 50                    | 23 $\pm$ 3    | 189 $\pm$ 17 | 16 $\pm$ 5  | 4 $\pm$ 4    | 11 $\pm$ 2    |
| 150                   | 17 $\pm$ 3    | 159 $\pm$ 7  | 12 $\pm$ 2  | 9 $\pm$ 3    | 10 $\pm$ 0    |
| 500                   | 20 $\pm$ 6    | 189 $\pm$ 29 | 14 $\pm$ 6  | 5 $\pm$ 4    | 11 $\pm$ 3    |
| 1500                  | 23 $\pm$ 7    | 185 $\pm$ 17 | 16 $\pm$ 2  | 8 $\pm$ 1    | 14 $\pm$ 3    |
| 5000                  | 19 $\pm$ 4    | 195 $\pm$ 8  | 10 $\pm$ 4  | 6 $\pm$ 3    | 12 $\pm$ 4    |
| Positive              | 1011 $\pm$ 36 | 998 $\pm$ 89 | 166 $\pm$ 8 | 169 $\pm$ 52 | 993 $\pm$ 161 |

Vehicle = Vehicle Control

Positive = Positive Control

Plating aliquot: 25  $\mu$ L

BioReliance

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**APPENDIX A**

**Historical Control Data**

| Historical Negative and Positive Control Values<br>1998 – 2000                                                                                                                                  |         |            |     |     |      |           |     |     |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|------------|-----|-----|------|-----------|-----|-----|------|
| revertants per plate                                                                                                                                                                            |         |            |     |     |      |           |     |     |      |
| Strain                                                                                                                                                                                          | Control | Activation |     |     |      |           |     |     |      |
|                                                                                                                                                                                                 |         | None       |     |     |      | Rat Liver |     |     |      |
|                                                                                                                                                                                                 |         | Mean       | SD  | Min | Max  | Mean      | SD  | Min | Max  |
| TA98                                                                                                                                                                                            | Neg     | 16         | 7   | 4   | 59   | 21        | 7   | 7   | 58   |
|                                                                                                                                                                                                 | Pos     | 425        | 206 | 21  | 1536 | 592       | 322 | 56  | 2454 |
| TA100                                                                                                                                                                                           | Neg     | 128        | 31  | 53  | 288  | 138       | 34  | 74  | 258  |
|                                                                                                                                                                                                 | Pos     | 568        | 159 | 129 | 1371 | 736       | 301 | 198 | 2871 |
| TA1535                                                                                                                                                                                          | Neg     | 12         | 5   | 1   | 45   | 12        | 4   | 1   | 42   |
|                                                                                                                                                                                                 | Pos     | 378        | 164 | 6   | 978  | 104       | 84  | 18  | 1640 |
| TA1537                                                                                                                                                                                          | Neg     | 6          | 3   | 0   | 30   | 7         | 3   | 1   | 29   |
|                                                                                                                                                                                                 | Pos     | 708        | 409 | 13  | 2786 | 88        | 106 | 12  | 2060 |
| WP2 <i>uvrA</i>                                                                                                                                                                                 | Neg     | 14         | 5   | 4   | 48   | 16        | 6   | 4   | 115  |
|                                                                                                                                                                                                 | Pos     | 190        | 138 | 34  | 961  | 317       | 299 | 22  | 2632 |
| 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 B**

**Study Protocol**



H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay

QA  
APPROVED

DuPont-8867

Received by RA/OA 06-04-2001

Sponsor Project Number: DuPont-8867

BioReliance Study Number: AA52XK.502001.BTL

H-25170: Bacterial Reverse Mutation Test  
with an Independent Repeat Assay

1.0 PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test substance 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 Name: E.I. du Pont de Nemours and Company

2.2 Address: Stine Haskell Research Center  
DuPont Haskell Laboratory  
P.O. Box 50  
1090 Elkton Road  
Newark, DE 19714-0050

2.3 Representative: Maria Donner, Ph.D.  
Phone: 302-366-5251  
Fax: 302-366-5207  
Email: [maria.donner@usa.dupont.com](mailto:maria.donner@usa.dupont.com)

2.4 Sponsor Project No.: DuPont-8867

2.5 WR#: [REDACTED]

2.6 Haskell #: H-25170

2.7 Service Code: [REDACTED]

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1 Test Substance Name: [REDACTED]

3.2 Test Substance I.D.: H-25170 (to be used in the report title and text)

3.3 Controls: Negative: Test substance vehicle  
Positive: 9-aminoacridine  
2-aminoanthracene  
methyl methanesulfonate  
2-nitrofluorene  
sodium azide

**H-25170: Bacterial Reverse Mutation Test with  
an Independent Repeat Assay**

**DuPont-8867**

Sponsor Project Number: DuPont-8867

BioReliance Study Number: AA52XK.502001.BTL

**3.4 Test Substance Characterization**

Unless alternate arrangements are made, the testing facility at BioReliance will not perform analysis of the dosing solutions. The Sponsor will be directly responsible for determination and documentation of the analytical purity and composition of the test substance, and the stability and strength of the test substance in the solvent (or vehicle).

**3.5 Test Substance Retention Sample**

The retention of a reserve sample of the test substance will be the responsibility of 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: [swagner@bioreliance.com](mailto:swagner@bioreliance.com)

**5.0 PROPOSED STUDY DATES**

- 5.1 Experimental Start Date: 07-Dec-2001
- 5.2 Experimental Termination Date: 25-Jan-2002
- 5.3 Draft Report Date: 08-Feb-2002
- 5.4 Final Report Date: 2 weeks after Sponsor approves draft

**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          | -               | -                   | rfa                  | $\Delta$ <i>uvrB</i> | -        |

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an Independent Repeat Assay**

**DuPont-8867**

Sponsor Project Number: DuPont-8867

BioReliance Study Number: AA52XK.502001.BTL

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

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. 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

The test substance 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 substance, negative controls and positive controls will be plated in triplicate.

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an Independent Repeat Assay**

**DuPont-8867**

Sponsor Project Number: DuPont-8867

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**7.1 Solubility Determination**

Unless the Sponsor has indicated the test substance vehicle, a solubility determination will be conducted to determine the maximum soluble concentration or workable suspension up to a maximum of 50 mg/mL for aqueous vehicles and 500 mg/mL for organic vehicles. 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/soluble stock concentration, up to 50 mg/mL for aqueous vehicles and 500 mg/mL for organic vehicles.

**7.2 Preliminary Toxicity Assay to Select Dose Levels**

Selection of dose levels for the mutagenicity assay will be based upon the toxicity and precipitation profile of the test substance assessed in a preliminary toxicity assay. This preliminary assay will be conducted by exposing TA98, TA100, TA1535, TA1537 and WP2 *uvrA* to negative controls and to at least eight concentrations of test substance, one plate per dose level, in both the presence and absence of S9 activation. 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. In selecting dose levels for the 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 mutagenicity assay will be selected to give some indication of toxicity without exceeding 5 mg/plate. For freely soluble, nontoxic test substances, the highest dose level will be 5 mg/plate. For precipitating, nontoxic test substances, the highest dose level will 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 substance dose level is less than 5 mg/plate and this dose level is nontoxic.

**7.3 Frequency and Route of Administration**

The test system will be exposed to the test substance 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).

After the data generated in the first assay have been evaluated, the mutagenicity assay will be repeated. The dose levels used in the second assay will be the same as those used in the first assay unless the Study Director determines that the dose levels should be changed due to an equivocal response, excessive cytotoxicity or

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excessive precipitate. If the Sponsor is aware of specific metabolic requirements (e.g., azo compounds), this information will be utilized in designing the assay. (e.g., activation system or treatment method). This guidance is based on the OECD Guideline 471 (adopted July 1997 and published February 1998) and ICH Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (1997).

#### 7.4 Controls

##### 7.4.1 Positive Controls

All combinations of positive controls and tester strains plated concurrently with the assay are listed below:

| Strain                    | S9 Activation | 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                    |

##### 7.4.2 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.

##### 7.4.3 Sterility Controls

The most concentrated test substance dilution and the Sham and S9 mixes will be checked for sterility.

#### 7.5 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 approximately

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-70°C until used. Each batch of S9 homogenate will be assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenzanthracene 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<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.

**7.6 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<sup>9</sup> cells/mL.

**7.7 Test System Identification**

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

**7.8 Test Substance Preparation**

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

**7.9 Treatment of Test System**

One half milliliter (0.5 mL) of S9 mix or Sham mix, 100  $\mu$ L of tester strain and 50  $\mu$ L of vehicle, test substance 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  $\mu$ L of test substance/vehicle/positive control will be plated. The mixture will be vortex mixed and overlaid onto the surface of 25 mL of minimal bottom agar.

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After the overlay has solidified, the plates will be inverted and incubated for approximately 48 to 72 hours at  $37\pm 2^\circ\text{C}$ . Plates that are not counted immediately following the incubation period will be stored at  $2-8^\circ\text{C}$ .

**7.10 Scoring**

The condition of the bacterial background lawn will be evaluated for evidence of test substance 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.

**7.11 Tester Strain Verification**

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

**8.0 CRITERIA FOR DETERMINATION OF A VALID TEST**

The following criteria must be met for the 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 Spontaneous Revertant Background Frequency**

Based on historical control data, all tester strain cultures must exhibit characteristic number of spontaneous revertants per plate in the negative controls (vehicle). 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.

**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.

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**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 negative 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 substance 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 substance 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 negative control value (vehicle).

**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 negative control value (vehicle).

**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. The report will include:

- Test Substance: 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 substance, if known.
- Solvent/Vehicle: justification for choice of vehicle; solubility and stability of test substance in solvent/vehicle, if known.
- Strains: strains used; number of cells/mL per culture; strain characteristics.

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- Test conditions: amount of test substance 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, where possible; statistical analysis, if any; concurrent negative and positive control data means and standard deviations; historical negative and positive control data with ranges, means and standard deviation.
- Discussion of results.
- Conclusion.

#### 11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports will be maintained according to Standard Operating Procedure [REDACTED] by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Bruschart 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.

#### 12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Assay), 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 performed in compliance with the provisions of the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (GLPs). The protocol, an in-process phase, the raw data, and report(s) will be audited 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. The in-process inspection will be performed to audit the critical assay procedures and systems supporting the assay. A signed QA statement will be included in the final report. This statement will list the system phases inspected during the previous quarter or the study-specific phases, 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 or control substances or their mixtures.

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Unless arrangements are made to the contrary, unused dosing solutions will be disposed of following administration to the test system and all residual test substance will be disposed of following finalization of the 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.

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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.

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 APPROVAL**

|                                                               |                            |
|---------------------------------------------------------------|----------------------------|
| <u>Maria Donner</u><br>Sponsor Representative                 | <u>03-DEC-2001</u><br>Date |
| <u>Maria Donner</u><br>(Print or Type Name)                   |                            |
| <u>Valentine D. Wagner, III</u><br>BioReliance Study Director | <u>5 Dec 2001</u><br>Date  |
| <u>Richard J</u><br>BioReliance Study Management              | <u>06 Dec 2001</u><br>Date |



**APPENDIX C**

**Information for Japanese Regulatory Agencies**

Report of Results of Reverse-Mutation Test in Bacteria

1. Tester Strains

(1) Procurement

| Strain                   | Obtained from                                                               | Date obtained    | Date inspected the strain lot in storage                             |
|--------------------------|-----------------------------------------------------------------------------|------------------|----------------------------------------------------------------------|
| TA98                     | Dr. Bruce Ames<br>University of California, Berkeley                        | 10 November 1998 | The genetic markers for each culture are confirmed on the day of use |
| TA100                    |                                                                             | 11 August 1998   |                                                                      |
| TA1535                   |                                                                             |                  |                                                                      |
| TA1537                   |                                                                             |                  |                                                                      |
| TA1538                   |                                                                             |                  |                                                                      |
| TA97                     |                                                                             | 13 December 1990 |                                                                      |
| TA102                    |                                                                             | 14 November 1990 |                                                                      |
| WP2 <i>uvrA</i>          | National Collection of Industrial and Marine Bacteria<br>Aberdeen, Scotland | 1 July 1987      |                                                                      |
| WP2 <i>uvrA</i> (pKM101) |                                                                             |                  |                                                                      |
| WP2 (pKM101)             |                                                                             | 19 February 1993 |                                                                      |

(2) Storage

|                     |                             |
|---------------------|-----------------------------|
| Freezing method     | Large quantity              |
| Storage temperature | -70°C                       |
| Composition         | Bacterial suspension 1.0 mL |
|                     | DMSO 0.09 mL                |

2. S9 Mix

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

|                     |                 |                                                               |               |
|---------------------|-----------------|---------------------------------------------------------------|---------------|
| Made in-house       | Prepared on     | 17 October 2001 (Batch R654)<br>26 November 2001 (Batch R656) |               |
| Storage temperature | -70°C or colder | Name and model of                                             | So-Low, Model |

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|  |  |                   |          |
|--|--|-------------------|----------|
|  |  | storage apparatus | PR27-120 |
|--|--|-------------------|----------|

**(2) Preparation of S9**

| Animal used     |                                                        | Inducing substance                                   |                                  |
|-----------------|--------------------------------------------------------|------------------------------------------------------|----------------------------------|
| Species, Strain | Rattus norvegicus, Sprague<br>Dawley                   | Name                                                 | Aroclor 1254                     |
| Sex             | Male                                                   | Administration<br>method                             | intraperitoneal                  |
| Age (in weeks)  | 8 (Batch R654)<br>9 (Batch R656)                       | Administration<br>period and amount<br>(g/kg-weight) | 5 days, 0.5 gm/kg<br>body weight |
| Weight          | 181 to 198 g (Batch R654)<br>187 to 271 g (Batch R656) |                                                      |                                  |

**3. Preparation of Test Substance Solution**

| Solvent used                                                            |                             |           |                            |
|-------------------------------------------------------------------------|-----------------------------|-----------|----------------------------|
| Name                                                                    | Manufacturer                | Lot No.   | Grade and/or<br>Purity (%) |
| Tetrahydrofuran<br>(CAS No. 109-99-9)                                   | Aldrich Chemical<br>Company | J100352H1 | Gold Label,<br>99.9%       |
| Stability of test substance in<br>the solvent                           | Unknown                     |           |                            |
| Method of suspension when<br>test substance is difficult to<br>dissolve | Not applicable              |           |                            |

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**4. Conditions of Pre-culture**

| Nutrient broth                                                          | Name                                 | Manufacturer | Lot No.                      |
|-------------------------------------------------------------------------|--------------------------------------|--------------|------------------------------|
|                                                                         | Oxoid Nutrient Broth No. 2           | Oxoid Ltd.   | CH,-B=232622<br>CH,-B=210287 |
| Period of pre-culture                                                   | 12±1 hours                           |              |                              |
| Storage time and temp. from inoculation to beginning of shaking culture | 2 to 5 hours at ambient temperature  |              |                              |
| Storage time and temp. from end of culture to use for test              | <8 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.      | 1000J3DKSQ       |

**(2) Minimum Glucose Agar**

|               |                             |              |                  |
|---------------|-----------------------------|--------------|------------------|
| Made in-house | Agar                        | Name         | BBL Select       |
|               |                             | Manufacturer | Becton Dickinson |
|               |                             | Lot No.      | 1000J3DKSQ       |
|               | Volume of agar plate medium |              | 25 mL            |

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6. Test Results - Judgement of the results

| Judgement                                                                                                                                                                          | Negative |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Reason for judgement and referential matters:<br><br>No positive response was observed with any of the tester strains in the presence and 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. |
|----------------------------------------------------------------------------------------------------------------------------------------------|