TRADE SECRET

Study Title

[redacted]Bacterial Reverse Mutation Test with an Independent Repeat Assay

Authors
Valentine O. Wagner, III, M.S.
Michelle L. Klug, B.S.

Report Completion Date

November 16, 2000

Performing Laboratory
BioReliance
9630 Medical Center Drive
Rockville, MD 20850

for

E. I. du Pont de Nemours and Company
Stine Haskell Research Center
DuPont Haskell Laboratory
P.O. Box 50
1090 Elkton Road
Newark, DE 19714-0050

Performing Laboratory Study Number
AA34BK.502001.BTL

DuPont Project ID
DuPont-4670

Work Request Number
[redacted]

Service Code
[redacted]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study No. AA34BK.502001.BTL was conducted in compliance with 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 control substances have not been determined by the testing facility. The control substances have been characterized as per the Certificates of Analysis on file with the testing facility.

Analyses to determine the uniformity (as applicable) or concentration of the test or control mixtures were not performed by the testing facility. The Sponsor has not performed these analyses.

The stability of the test or control substances in the test or control mixtures has not been determined by the testing facility. The Sponsor has not performed these analyses.

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

BioReliance Study Director: Valentine O. Wagner, III
Valentine O. Wagner, III, M.S. — 16 Nov 2000 — Date

BioReliance Study Management: [signature]
Richard H.C. San, Ph.D. — 16 Nov 2000 — Date

DuPont Registration Representative: _____ — _____ — Date

TRADE SECRET

Study Title

[redacted] Bacterial Reverse Mutation Test with an Independent Repeat Assay

Authors
Valentine O. Wagner, III, M.S.
Michelle L. Klug, B.S.

Report Completion Date

November 16, 2000

Performing Laboratory
BioReliance
9630 Medical Center Drive
Rockville, MD 20850

for

E. I. du Pont de Nemours and Company
Stine Haskell Research Center
DuPont Haskell Laboratory
P.O. Box 50
1090 Elkton Road
Newark, DE 19714-0050

Performing Laboratory Study Number
AA34BK.502001.BTL

DuPont Project ID
DuPont-4670

Work Request Number
[redacted]

Service Code
[redacted]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study No. AA34BK.502001.BTL was conducted in compliance with 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 control substances have not been determined by the testing facility. The control substances have been characterized as per the Certificates of Analysis on file with the testing facility.

Analyses to determine the uniformity (as applicable) or concentration of the test or control mixtures were not performed by the testing facility. The Sponsor has not performed these analyses.

The stability of the test or control substances in the test or control mixtures has not been determined by the testing facility. The Sponsor has not performed these analyses.

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

BioReliance Study Director: Valentine O. Wagner, III — 16 Nov 2000
Valentine O. Wagner, III, M.S. — Date

BioReliance Study Management: [signature] — 16 Nov 2000
Richard H.C. San, Ph.D. — Date

DuPont Registration Representative: _____ — _____
Date

Quality Assurance Statement

Study Title: [redacted] BACTERIAL REVERSE MUTATION TEST WITH AN INDEPENDENT REPEAT ASSAY

Study Number: AA34BK.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	25-Aug-00 - 25-Aug-00 To Study Dir 25-Aug-00 To Mgmt 25-Aug-00
Phase	Protocol Review
Inspect On	12-Sep-00 - 12-Sep-00 To Study Dir 12-Sep-00 To Mgmt 14-Sep-00
Phase	Preparation of S9 mixture
Inspect On	30-Oct-00 - 30-Oct-00 To Study Dir 30-Oct-00 To Mgmt 01-Nov-00
Phase	Draft Report
Inspect On	14-Nov-00 - 14-Nov-00 To Study Dir 14-Nov-00 To Mgmt 14-Nov-00
Phase	Draft to Final Report

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

Diane Brecha

Diane Brecha, B.S.
QUALITY ASSURANCE

16 Nov 2000
DATE

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 — 16 Nov 2000

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

Date

Approved by
Sponsor Study Monitor: Maria Donner — 15 Nov 2000

Maria Donner, Ph.D.
Senior Research Scientist

Date

TABLE OF CONTENTS

STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes:
- [redacted]
- H-24547

Haskell Number: 24547

Composition: [redacted]

Known Impurities: Not supplied by the sponsor

Physical Characteristics: Amber sticky gum

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: August 24, 2000 / (see report cover page)

In-Life Initiated/Completed: August 29, 2000 / October 04, 2000

SUMMARY

The test substance, H-24547, was tested in the bacterial reverse mutation test using *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *E. coli* tester strain WP2 *uvr*A 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 assay. The second phase, the mutagenicity test (initial and independent repeat assays), was used to evaluate the mutagenic potential of the test substance.

Acetone was selected as the solvent of choice based on solubility of the test substance and compatibility with the target cells. The test substance was a clear solution in acetone at ≤300 mg/mL.

In the preliminary toxicity test, the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 100 mg/mL and a 50 µL plating aliquot. Neither precipitate nor 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 response was observed. In the initial mutagenicity assay, the dose levels tested were 5000, 3333, 1000, 333 and 100 µg per plate. Neither precipitate nor toxicity was observed. In the independent repeat assay, the dose levels tested were 5000, 1800, 600, 200 and 75 µg per plate. Neither precipitate nor toxicity was observed.

All criteria for a valid study were met as described in the protocol. The results of the [redacted] Bacterial Reverse Mutation Test with an Independent Repeat Assay indicate that, under the conditions of this study, the test substance did not cause a positive mutagenic response with any of the tester strains in the presence and 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* WP2 *uvr*A in the presence and absence of S9 activation.

CHARACTERIZATION OF TEST AND CONTROL SUBSTANCES

The test substance, H-24547, was received by BioReliance on 17 August 2000 and was assigned the code number AA34BK. The test substance was characterized by the Sponsor as an amber sticky gum that should be stored at ambient temperature. An expiration date of 15 August 2003 was provided. Upon receipt, the test substance was described as a yellow solid and was stored at room temperature, protected from exposure to light and moisture.

The vehicle used to deliver H-24547 to the test system was acetone, (CAS# 67-64-1), obtained from Fisher Scientific.

Positive controls plated concurrently with the mutagenicity test are listed below:

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	+	2-aminoanthracene (Sigma Chemical Co.)	1.0
WP2 *uvr*A			10
TA98	–	2-nitrofluorene (Aldrich Chemical Co., Inc.)	1.0
TA100, TA1535		sodium azide (Sigma Chemical Co.)	1.0
TA1537		9-aminoacridine (Sigma Chemical Co.)	75
WP2 *uvr*A		methyl methanesulfonate (Aldrich Chemical Co., Inc.)	1,000

To determine 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 assay.

MATERIALS AND METHODS

Test System

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al.* (1975), and *Escherichia coli* tester strain WP2 *uvr*A. *Salmonella* tester strains were received on 11 August 1998 and 10 November 1998 directly from Dr. Bruce Ames, University of California, Berkeley. *E. coli* was received on 01 July 1987 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 greater than or equal to 0.3×10^9 cells per milliliter. The actual titers were determined by viable count assays 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 and with the International Conference on Harmonisation of Technical Requirements for 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 prepared by BioReliance was batch prepared on 22 June 2000. The S9 purchased from MolTox was batch prepared 17 December 1999. All S9 batches were stored at ≤-70°C until used. Each bulk preparation of S9 was assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

The S9 mix was prepared immediately before its use and contained 10% S9, 5 mM glucose-6-phosphate, 4 mM β-nicotinamide-adenine dinucleotide phosphate, 8 mM $MgCl_2$ 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 one or more of the following: water, dimethyl sulfoxide (DMSO), ethanol and acetone. 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 500 mg/mL.

Preliminary Toxicity Test

The preliminary toxicity test was used to establish the dose-range over which the test substance would be assayed. Vehicle and ten dose levels of the test substance (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg per plate) were plated. Each dose level was plated on a single plate, with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective minimal agar in both the presence and absence of rat liver S9 activation.

Mutagenicity Test

The mutagenicity test initial assay (Experiment B1) and independent repeat assay (Experiment B2) was used to evaluate the mutagenic potential of the test substance. A minimum of five dose levels of test substance along with appropriate vehicle and positive controls were plated with tester strains TA98, TA100, TA1535, TA1537 and WP2 *uvr*A in the presence and absence of rat liver S9 activation. All dose levels of test substance, vehicle controls and positive controls were plated in triplicate.

Plating and Scoring Procedures

In both the preliminary toxicity test and the mutagenicity test, the test system was exposed to the test substance via the plate incorporation methodology as described by Ames *et* al. (1975) and updated by Maron and Ames (1983).

On the day of its use, minimal top agar, containing 0.8 % agar (W/V) and 0.5 % NaCl (W/V), was melted and supplemented with L-histidine, D-biotin and L-tryptophan solution to a final concentration of 50 µM each. Top agar not used with S9 or Sham mix was supplemented with 25 mL of water for each 100 mL of minimal top agar. For the preparation of media and reagents, all references to water imply sterile, deionized water produced by the Milli-Q Reagent

Water System. Bottom agar was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) containing 1.5 % (W/V) agar. Nutrient bottom agar was Vogel-Bonner minimal medium E containing 1.5 % (W/V) agar and supplemented with 2.5 % (W/V) Oxoid Nutrient Broth No. 2 (dry powder). Nutrient Broth was Vogel-Bonner salt solution supplemented with 2.5 % (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

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

Test substance dilutions were prepared immediately before use. One-half (0.5) milliliter of S9 or Sham mix, 100 µL of tester strain and 50 µL of vehicle or test substance were added to 2.0 mL of molten selective top agar at 45±2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

The condition of the bacterial background lawn was evaluated for evidence of test 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	Severely 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 ≥90% of the plate.
6	Obscured by Precipitate	The background bacterial lawn cannot be accurately evaluated due to microscopic test substance precipitate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than 10% of the revertant colony count (e.g., ≤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., >3 particles on a plate with 30 revertants.)

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 test was the preliminary toxicity test or the plate exhibited toxicity. Plates with sufficient test substance precipitate to interfere with automated colony counting were counted manually.

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 with a minimum of two increasing concentrations of test substance. Data sets for 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 three times the mean vehicle control value. Data sets for strains TA98, TA100 and

WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two 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 *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A 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); and WP2 *uvr*A (10 – 60). To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/mL. The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels are 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 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 reduction in the background lawn.

Archives

The study raw data, protocol and all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850.

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

Acetone was selected as the solvent of choice based on solubility of the test substance and compatibility with the target cells. The test substance was a soluble and clear solution in acetone at ≤300 mg/mL.

Preliminary Toxicity Test

The results of the preliminary toxicity test are presented in Tables 1 through 5. In the preliminary toxicity test, the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 100 mg/mL and a 50 µL plating aliquot. Neither precipitate nor 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. In the mutagenicity test, no positive response was observed. In the initial mutagenicity assay, the dose levels tested were 5000, 3333, 1000, 333 and 100 µg per plate. Neither precipitate nor toxicity was observed. In the independent repeat assay, the dose levels tested were 5000, 1800, 600, 200 and 75 µg per plate. Neither precipitate nor toxicity was observed.

No positive responses were observed with any of the tester strains in the presence and absence of S9 activation in either the initial mutagenicity assay or in the independent repeat assay.

CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the [redacted] Bacterial Reverse Mutation Test with an Independent Repeat Assay indicate that, under the conditions of this study, the test substance did not cause a positive mutagenic response with any of the tester strains in the presence and 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.

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.

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

OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), adopted July 1997.

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

Bacterial Mutation Test
Preliminary Toxicity Assay

Table 1

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA98
Experiment No. : A1
Date Plated : 29 Aug 2000
Vehicle : acetone
Plating Aliquot : 50 µL

Test Substance Concentration µg per plate	With S9 Activation Revertants per plate	With S9 Activation Background Lawn	Without S9 Activation Revertants per plate	Without S9 Activation Background Lawn
Vehicle	21	1	22	1
6.7	25	1	26	1
10	29	1	19	1
33	24	1	17	1
67	24	1	19	1
100	19	1	25	1
333	17	1	10	1
667	15	1	17	1
1000	20	1	18	1
3333	20	1	22	1
5000	27	1	21	1

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Severely reduced
4=Extremely reduced; 5=Absent; 6=Obscured by precipitate
NP=Non-Interfering precipitate; IP= Interfering precipitate
C=Contaminated

Bacterial Mutation Test
Preliminary Toxicity Assay

Table 2

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA100
Experiment No. : A1
Date Plated : 29 Aug 2000
Vehicle : acetone
Plating Aliquot : 50 µL

Test Substance Concentration µg per plate	With S9 Activation Revertants per plate	With S9 Activation Background Lawn	Without S9 Activation Revertants per plate	Without S9 Activation Background Lawn
Vehicle	91	1	129	1
6.7	108	1	124	1
10	97	1	96	1
33	125	1	87	1
67	117	1	115	1
100	110	1	89	1
333	118	1	89	1
667	132	1	134	1
1000	131	1	128	1
3333	114	1	118	1
5000	121	1	161	1

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

Bacterial Mutation Test
Preliminary Toxicity Assay

Table 3

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1535
Experiment No. : A1
Date Plated : 29 Aug 2000
Vehicle : acetone
Plating Aliquot : 50 µL

Test Substance Concentration µg per plate	With S9 Activation Revertants per plate	With S9 Activation Background Lawn	Without S9 Activation Revertants per plate	Without S9 Activation Background Lawn
Vehicle	9	1	13	1
6.7	10	1	16	1
10	13	1	15	1
33	11	1	14	1
67	10	1	8	1
100	15	1	13	1
333	12	1	11	1
667	10	1	13	1
1000	16	1	16	1
3333	18	1	9	1
5000	16	1	12	1

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

Bacterial Mutation Test
Preliminary Toxicity Assay

Table 4

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1537
Experiment No. : A1
Date Plated : 29 Aug 2000
Vehicle : acetone
Plating Aliquot : 50 µL

Test Substance Concentration µg per plate	With S9 Activation Revertants per plate	With S9 Activation Background Lawn	Without S9 Activation Revertants per plate	Without S9 Activation Background Lawn
Vehicle	7	1	6	1
6.7	8	1	7	1
10	5	1	7	1
33	7	1	9	1
67	4	1	4	1
100	6	1	10	1
333	5	1	8	1
667	7	1	2	1
1000	11	1	5	1
3333	8	1	2	1
5000	4	1	6	1

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

Bacterial Mutation Test
Preliminary Toxicity Assay

Table 5

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : WP2 *uvrA*
Experiment No. : A1
Date Plated : 29 Aug 2000
Vehicle : acetone
Plating Aliquot : 50 µL

Test Substance Concentration µg per plate	With S9 Activation Revertants per plate	With S9 Activation Background Lawn	Without S9 Activation Revertants per plate	Without S9 Activation Background Lawn
Vehicle	14	1	12	1
6.7	16	1	9	1
10	12	1	15	1
33	11	1	11	1
67	14	1	17	1
100	15	1	12	1
333	11	1	10	1
667	10	1	14	1
1000	13	1	10	1
3333	13	1	13	1
5000	15	1	9	1

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

Bacterial Mutation Test

Table 6

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA98
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.1 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	28	1		
	02	21	1		
	03	44	1	31	12
100	01	37	1		
	02	19	1		
	03	25	1	27	9
333	01	37	1		
	02	34	1		
	03	41	1	37	4
1000	01	27	1		
	02	31	1		
	03	28	1	29	2
3333	01	28	1		
	02	31	1		
	03	25	1	28	3
5000	01	40	1		
	02	21	1		
	03	30	1	30	10
Positive Control 2-nitrofluorene 1.0 µg per plate					
	01	391	1		
	02	283	1		
	03	337	1	337	54

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

Bacterial Mutation Test

Table 7

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA98
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.1 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	27	1		
	02	34	1		
	03	27	1	29	4
100	01	27	1		
	02	29	1		
	03	26	1	27	2
333	01	42	1		
	02	34	1		
	03	37	1	38	4
1000	01	38	1		
	02	23	1		
	03	30	1	30	8
3333	01	31	1		
	02	34	1		
	03	21	1	29	7
5000	01	30	1		
	02	36	1		
	03	25	1	30	6
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	385	1		
	02	457	1		
	03	313	1	385	72

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

Bacterial Mutation Test

Table 8

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA100
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.3 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	95	1		
	02	81	1		
	03	111	1	96	15
100	01	97	1		
	02	120	1		
	03	79	1	99	21
333	01	115	1		
	02	75	1		
	03	82	1	91	21
1000	01	103	1		
	02	82	1		
	03	97	1	94	11
3333	01	83	1		
	02	91	1		
	03	93	1	89	5
5000	01	88	1		
	02	110	1		
	03	116	1	105	15
Positive Control sodium azide 1.0 µg per plate					
	01	341	1		
	02	320	1		
	03	426	1	362	56

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

Bacterial Mutation Test

Table 9

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA100
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.3 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	88	1		
	02	96	1		
	03	107	1	97	10
100	01	78	1		
	02	113	1		
	03	96	1	96	18
333	01	96	1		
	02	91	1		
	03	81	1	89	8
1000	01	89	1		
	02	102	1		
	03	105	1	99	9
3333	01	79	1		
	02	81	1		
	03	61	1	74	11
5000	01	86	1		
	02	87	1		
	03	82	1	85	3
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	321	1		
	02	971	1		
	03	445	1	579	345

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

Bacterial Mutation Test

Table 10

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1535
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.7 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	14	1		
	02	18	1		
	03	13	1	15	3
100	01	17	1		
	02	23	1		
	03	25	1	22	4
333	01	15	1		
	02	20	1		
	03	15	1	17	3
1000	01	17	1		
	02	21	1		
	03	10	1	16	6
3333	01	13	1		
	02	16	1		
	03	14	1	14	2
5000	01	19	1		
	02	15	1		
	03	16	1	17	2
Positive Control sodium azide 1.0 µg per plate					
	01	401	1		
	02	262	1		
	03	281	1	315	75

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

Bacterial Mutation Test

Table 11

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1535
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.7 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	19	1		
	02	8	1		
	03	17	1	15	6
100	01	11	1		
	02	9	1		
	03	14	1	11	3
333	01	13	1		
	02	12	1		
	03	9	1	11	2
1000	01	17	1		
	02	11	1		
	03	14	1	14	3
3333	01	15	1		
	02	13	1		
	03	11	1	13	2
5000	01	12	1		
	02	13	1		
	03	21	1	15	5
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	79	1		
	02	69	1		
	03	71	1	73	5

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

Bacterial Mutation Test

Table 12

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1537
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.1 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	5	1		
	02	7	1		
	03	9	1	7	2
100	01	7	1		
	02	5	1		
	03	C		6	1
333	01	7	1		
	02	4	1		
	03	6	1	6	2
1000	01	9	1		
	02	9	1		
	03	3	1	7	3
3333	01	9	1		
	02	10	1		
	03	5	1	8	3
5000	01	9	1		
	02	6	1		
	03	2	1	6	4
Positive Control 9-aminoacridine 75 µg per plate					
	01	1070	1		
	02	978	1		
	03	691	1	913	198

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Severely reduced
4=Extremely reduced; 5=Absent; 6=Obscured by precipitate
NP=Non-Interfering precipitate; IP= Interfering precipitate
C=Contaminated

Bacterial Mutation Test

Table 13

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1537
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 1.1 X 10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	6	1		
	02	4	1		
	03	5	1	5	1
100	01	4	1		
	02	3	1		
	03	7	1	5	2
333	01	5	1		
	02	5	1		
	03	8	1	6	2
1000	01	6	1		
	02	3	1		
	03	8	1	6	3
3333	01	2	1		
	02	6	1		
	03	5	1	4	2
5000	01	4	1		
	02	5	1		
	03	6	1	5	1
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	80	1		
	02	84	1		
	03	113	1	92	18

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

Bacterial Mutation Test

Table 14

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : WP2 *uvrA*
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 3.0×10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	12	1		
	02	16	1		
	03	19	1	16	4
100	01	11	1		
	02	15	1		
	03	10	1	12	3
333	01	12	1		
	02	16	1		
	03	22	1	17	5
1000	01	13	1		
	02	14	1		
	03	14	1	14	1
3333	01	12	1		
	02	10	1		
	03	19	1	14	5
5000	01	13	1		
	02	14	1		
	03	8	1	12	3
Positive Control methyl methanesulfonate 1000 µg per plate					
	01	73	1		
	02	69	1		
	03	72	1	71	2

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

Bacterial Mutation Test

Table 15

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : WP2 *uvrA*
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B1
Cells Seeded : 3.0×10^8
Date Plated : 12 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	11	1		
	02	16	1		
	03	14	1	14	3
100	01	16	1		
	02	12	1		
	03	11	1	13	3
333	01	10	1		
	02	15	1		
	03	12	1	12	3
1000	01	12	1		
	02	9	1		
	03	10	1	10	2
3333	01	11	1		
	02	13	1		
	03	10	1	11	2
5000	01	11	1		
	02	13	1		
	03	12	1	12	1
Positive Control 2-aminoanthracene 10 µg per plate					
	01	94	1		
	02	70	1		
	03	171	1	112	53

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

Bacterial Mutation Test

Table 16

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA98
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 2.4 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	16	1		
	02	17	1		
	03	23	1	19	4
75	01	17	1		
	02	12	1		
	03	15	1	15	3
200	01	12	1		
	02	14	1		
	03	20	1	15	4
600	01	14	1		
	02	16	1		
	03	16	1	15	1
1800	01	18	1		
	02	14	1		
	03	16	1	16	2
5000	01	17	1		
	02	16	1		
	03	18	1	17	1
Positive Control 2-nitrofluorene 1.0 µg per plate					
	01	191	1		
	02	190	1		
	03	170	1	184	12

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

Bacterial Mutation Test

Table 17

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA98
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 2.4 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	19	1		
	02	16	1		
	03	20	1	18	2
75	01	18	1		
	02	11	1		
	03	15	1	15	4
200	01	14	1		
	02	13	1		
	03	21	1	16	4
600	01	14	1		
	02	9	1		
	03	6	1	10	4
1800	01	13	1		
	02	11	1		
	03	12	1	12	1
5000	01	13	1		
	02	14	1		
	03	17	1	15	2
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	118	1		
	02	191	1		
	03	293	1	201	88

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

Bacterial Mutation Test

Table 18

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA100
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 1.8 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	124	1		
	02	131	1		
	03	142	1	132	9
75	01	125	1		
	02	120	1		
	03	110	1	118	8
200	01	134	1		
	02	123	1		
	03	88	1	115	24
600	01	123	1		
	02	127	1		
	03	120	1	123	4
1800	01	146	1		
	02	122	1		
	03	132	1	133	12
5000	01	138	1		
	02	133	1		
	03	140	1	137	4
Positive Control sodium azide 1.0 µg per plate					
	01	573	1		
	02	613	1		
	03	587	1	591	20

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

Bacterial Mutation Test

Table 19

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA100
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 1.8 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	98	1		
	02	92	1		
	03	110	1	100	9
75	01	85	1		
	02	97	1		
	03	92	1	91	6
200	01	121	1		
	02	88	1		
	03	117	1	109	18
600	01	102	1		
	02	98	1		
	03	120	1	107	12
1800	01	118	1		
	02	86	1		
	03	110	1	105	17
5000	01	124	1		
	02	105	1		
	03	114	1	114	10
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	604	1		
	02	571	1		
	03	520	1	565	42

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

Bacterial Mutation Test

Table 20

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1535
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 2.5 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	12	1		
	02	7	1		
	03	10	1	10	3
75	01	11	1		
	02	12	1		
	03	17	1	13	3
200	01	14	1		
	02	12	1		
	03	13	1	13	1
600	01	11	1		
	02	12	1		
	03	12	1	12	1
1800	01	14	1		
	02	13	1		
	03	13	1	13	1
5000	01	15	1		
	02	12	1		
	03	12	1	13	2
Positive Control sodium azide 1.0 µg per plate					
	01	364	1		
	02	245	1		
	03	268	1	292	63

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

Bacterial Mutation Test

Table 21

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1535
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 2.5×10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	10	1		
	02	8	1		
	03	11	1	10	2
75	01	12	1		
	02	13	1		
	03	16	1	14	2
200	01	8	1		
	02	9	1		
	03	8	1	8	1
600	01	6	1		
	02	8	1		
	03	11	1	8	3
1800	01	9	1		
	02	7	1		
	03	13	1	10	3
5000	01	13	1		
	02	9	1		
	03	15	1	12	3
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	93	1		
	02	133	1		
	03	100	1	109	21

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

Bacterial Mutation Test

Table 22

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1537
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 0.8 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	5	1		
	02	6	1		
	03	5	1	5	1
75	01	7	1		
	02	5	1		
	03	6	1	6	1
200	01	6	1		
	02	4	1		
	03	8	1	6	2
600	01	4	1		
	02	4	1		
	03	5	1	4	1
1800	01	4	1		
	02	3	1		
	03	4	1	4	1
5000	01	7	1		
	02	6	1		
	03	7	1	7	1
Positive Control 9-aminoacridine 75 µg per plate					
	01	215	1		
	02	196	1		
	03	167	1	193	24

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

Bacterial Mutation Test

Table 23

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : TA1537
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 0.8 X 10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	5	1		
	02	11	1		
	03	7	1	8	3
75	01	6	1		
	02	4	1		
	03	6	1	5	1
200	01	4	1		
	02	7	1		
	03	7	1	6	2
600	01	5	1		
	02	3	1		
	03	6	1	5	2
1800	01	7	1		
	02	6	1		
	03	6	1	6	1
5000	01	6	1		
	02	5	1		
	03	2	1	4	2
Positive Control 2-aminoanthracene 1.0 µg per plate					
	01	89	1		
	02	82	1		
	03	68	1	80	11

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

Bacterial Mutation Test

Table 24

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : WP2 *uvrA*
Liver Microsomes : None
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 3.5×10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	12	1		
	02	14	1		
	03	15	1	14	2
75	01	15	1		
	02	13	1		
	03	11	1	13	2
200	01	12	1		
	02	11	1		
	03	11	1	11	1
600	01	10	1		
	02	11	1		
	03	13	1	11	2
1800	01	12	1		
	02	15	1		
	03	18	1	15	3
5000	01	12	1		
	02	16	1		
	03	16	1	15	2
Positive Control methyl methanesulfonate 1000 µg per plate					
	01	128	1		
	02	94	1		
	03	96	1	106	19

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

Bacterial Mutation Test

Table 25

Test Substance Id: [redacted]

Study Number : AA34BK.502001.BTL
Strain : WP2 *uvrA*
Liver Microsomes : Rat liver S9
Vehicle : acetone
Plating Aliquot : 50 µL

Experiment No : B2
Cells Seeded : 3.5×10^8
Date Plated : 29 Sep 2000

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	01	16	1		
	02	12	1		
	03	13	1	14	2
75	01	19	1		
	02	10	1		
	03	12	1	14	5
200	01	11	1		
	02	13	1		
	03	11	1	12	1
600	01	11	1		
	02	13	1		
	03	11	1	12	1
1800	01	14	1		
	02	14	1		
	03	10	1	13	2
5000	01	15	1		
	02	12	1		
	03	9	1	12	3
Positive Control 2-aminoanthracene 10 µg per plate					
	01	145	1		
	02	153	1		
	03	186	1	161	22

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

Bacterial Mutation Test
Summary of Results

Table 26

Test Substance Id : [redacted]

Study Number : AA34BK.502001.BTL Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	31 ± 12	96 ± 15	15 ± 3	7 ± 2	16 ± 4
100	27 ± 9	99 ± 21	22 ± 4	6 ± 1	12 ± 3
333	37 ± 4	91 ± 21	17 ± 3	6 ± 2	17 ± 5
1000	29 ± 2	94 ± 11	16 ± 6	7 ± 3	14 ± 1
3333	28 ± 3	89 ± 5	14 ± 2	8 ± 3	14 ± 5
5000	30 ± 10	105 ± 15	17 ± 2	6 ± 4	12 ± 3
Positive	337 ± 54	362 ± 56	315 ± 75	913 ± 198	71 ± 2

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	29 ± 4	97 ± 10	15 ± 6	5 ± 1	14 ± 3
100	27 ± 2	96 ± 18	11 ± 3	5 ± 2	13 ± 3
333	38 ± 4	89 ± 8	11 ± 2	6 ± 2	12 ± 3
1000	30 ± 8	99 ± 9	14 ± 3	6 ± 3	10 ± 2
3333	29 ± 7	74 ± 11	13 ± 2	4 ± 2	11 ± 2
5000	30 ± 6	85 ± 3	15 ± 5	5 ± 1	12 ± 1
Positive	385 ± 72	579 ± 345	73 ± 5	92 ± 18	112 ± 53

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Bacterial Mutation Test
Summary of Results

Table 27

Test Substance Id : [redacted]

Study Number : AA34BK.502001.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	19 ± 4	132 ± 9	10 ± 3	5 ± 1	14 ± 2
75	15 ± 3	118 ± 8	13 ± 3	6 ± 1	13 ± 2
200	15 ± 4	115 ± 24	13 ± 1	6 ± 2	11 ± 1
600	15 ± 1	123 ± 4	12 ± 1	4 ± 1	11 ± 2
1800	16 ± 2	133 ± 12	13 ± 1	4 ± 1	15 ± 3
5000	17 ± 1	137 ± 4	13 ± 2	7 ± 1	15 ± 2
Positive	184 ± 12	591 ± 20	292 ± 63	193 ± 24	106 ± 19

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	18 ± 2	100 ± 9	10 ± 2	8 ± 3	14 ± 2
75	15 ± 4	91 ± 6	14 ± 2	5 ± 1	14 ± 5
200	16 ± 4	109 ± 18	8 ± 1	6 ± 2	12 ± 1
600	10 ± 4	107 ± 12	8 ± 3	5 ± 2	12 ± 1
1800	12 ± 1	105 ± 17	10 ± 3	6 ± 1	13 ± 2
5000	15 ± 2	114 ± 10	12 ± 3	4 ± 2	12 ± 3
Positive	201 ± 88	565 ± 42	109 ± 21	80 ± 11	161 ± 22

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

APPENDIX A

Historical Control Data

Historical Negative and Positive Control Values
1997 – 1999

revertants per plate

Strain	Control	Activation							
		None				Rat Liver			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA98	Neg	16	6	4	59	21	7	6	65
	Pos	397	216	21	1536	649	330	56	2454
TA100	Neg	128	29	53	288	144	30	62	258
	Pos	592	159	129	1920	785	300	106	2871
TA1535	Neg	11	5	1	45	12	5	1	42
	Pos	424	145	6	1024	98	79	8	1640
TA1537	Neg	6	3	0	30	7	3	1	29
	Pos	819	468	13	2786	102	128	2	1835
WP2 *uvr*A	Neg	16	5	3	48	17	6	2	115
	Pos	190	127	35	961	338	282	16	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

QA APPROVED 8/25/00

Received by RA/OA 25-Aug-2000

Sponsor Project Number: DuPont-4670

BioReliance Study Number: AA34BK.502001.BTL

[redacted] 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.
2.4	Sponsor Project No.:	DuPont-4670
2.5	WR#:	[redacted]
2.6	Haskell #:	H-24547
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-24547 (to be used in the report text)
3.3	Controls:	[redacted]

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.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

5.0 PROPOSED STUDY DATES

5.1	Experimental Start Date:	30-Aug-2000
5.2	Experimental Termination Date:	17-Oct-2000
5.3	Draft Report Date:	31-Oct-2000
5.4	Final Report Date:	8-Nov-2000

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).

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.502001.BTL

Genotype of the Strains Used for Mutagen Testing						
Histidine Mutation			Tryptophan Mutation	Additional Mutations		
hisG46	hisC3076	HisD3052	TrpE	LPS	Repair	R-factor
TA1535	TA1537	-	-	Rfa	ΔuvrB	-
TA100	-	TA98	-	Rfa	ΔuvrB	+R
-	-	-	WP2 uvrA	-	ΔuvrA	-

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.

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.502001.BTL

7.1 Solubility Determination

A solubility determination will be conducted to determine the maximum soluble concentration or workable suspension up to a maximum of 500 mg/mL. 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 500 mg/mL.

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 *uvr*A 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 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

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.502001.BTL

OECD Guideline 471 (adopted July 1997) 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)
Salmonella Strains WP2 uvrA	Rat	2-aminoanthracene	1.0
			10
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 uvrA		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 -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 β-nicotinamide-adenine dinucleotide phosphate, 8 mM $MgCl_2$ and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4. This mixture is referred to as S9 mix. Sham mix will be 100 mM phosphate buffer at pH 7.4.

Sponsor Project Number: DuPont-4670

BioReliance Study Number: AA34BK.502001.BTL

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 10^9 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 µL of tester strain and 50 µL 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 µL 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. After the overlay has solidified, the plates will be inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that are not counted immediately following the incubation period will be stored at 2-8°C.

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.

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.502001.BTL

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 *uvr*B mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the *uvr*A 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 *uvr*A, 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 0.3×10^9 cells per milliliter.

8.4 Positive Control Values

Each mean positive control value must exhibit at least a three 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

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.502001.BTL

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 three 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 two 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.
- 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.

Sponsor Project Number: DuPont-4670
BioReliance Study Number: AA34BK.502001.BTL

- Age and mean body weights of rats used to prepare S9

11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850.

12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline 471 for Testing of Chemicals (Genetic Toxicology: Bacterial Reverse Mutation Assay), Adopted 1997 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 EPA (TSCA), OECD, Japanese (MITI) and EEC Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.

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^+ 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.

Sponsor Project Number: DuPont-4670

BioReliance Study Number: AA34BK.502001.BTL

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 for Testing of Chemicals (Genetic Toxicology: Bacterial Reverse Mutation Assay), Adopted July 1997.

Wilcox, P., Naidoo, A., Wedd, D.J. and Gatehouse, D.G. (1990). Comparison of *Salmonella typhimurium* TA102 with *Escherichia coli* WP2 tester strains. Mutagenesis 5:285-291.

14.0 APPROVAL

Maria Donner — Sponsor Representative — 22 Aug 2000 — Date

Maria Donner — (Print or Type Name)

Valentine O. Wagner III — BioReliance Study Director — 24 Aug 2000 — Date

[signature] — BioReliance Study Management — 24 Aug 2000 — Date

APPENDIX C

Information for Japanese Regulatory Agencies

Report of Results of Reverse-Mutation Assay in Bacteria

1. General Items

Name of the new chemical substance (IUPAC nomenclature)					
Other name	Fluoroalkyl Urethane	Physicochemical properties of the new chemical substance	Molecular weight		
Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)			Appearance at ordinary temperature		
			Stability		
			Melting point		
			Boiling point		
			Vapor pressure		
			Partition coefficient		
Purity of the new chemical substance tested			Solubility		
			Degree of solubility	Water	
				DMSO	
Name and concentration of impurities	100.0 wt %			Acetone	300 mg/mL
				Other ( )	

[Remarks] Because physicochemical properties are reference materials, fill in spaces to extent possible.

1."Stability"-Fill in the stability for water, other solvents, heat, light, etc.
2."Vapor pressure"-Fill in the vapor pressure of the test substance at 25°C.
3."Partition coefficient"-Fill in the value, the temperature used and the name of the solvent used for the measurement.
4."Solubility"-Fill in such information as water-soluble, soluble in oil.
5."Degree of solubility"-Fill in the solubility at 25°C for each solvent.

2. Tester Strains

(1) Procurement

Strain	Obtained from	Date obtained	Date inspected the strain lot in storage
TA98	Dr. Bruce Ames 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 *uvr*A	National Collection of Industrial and Marine Bacteria Aberdeen, Scotland	1 July 1987	
WP2 *uvr*A (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

3. S9 Mix

(1) Source of S9

Made in-house	Prepared on	22 June 2000
Purchased from: MolTox	Batch 1030 prepared on	17 December 1999

(2) Storage Temperature, etc. of S9

Storage temperature	-70°C	Name and model of	So-Low, Model

		storage apparatus	PR27-120

(3) Preparation of S9

Animal used		Inducing substance	
Species, Strain	Rattus norvegicus, Sprague Dawley	Name	Aroclor 1254
Sex	Male	Administration method	intraperitoneal
Age (in weeks)	9	Administration period and amount (g/kg-weight)	5 days, 0.5 gm/kg body weight
Weight	234 to 252 g		

4. Positive Control Substance

(1) Positive control

Name	Manufacture	Lot No.	Grade	Purity (%)	Solvent used
9-Aminoacridine (9AAD)	Sigma Chemical Company	106F06681		>98 %	DMSO
2-Aminoanthracene (2AA)	Sigma Chemical Company	085H2508	Practical	>95 %	DMSO
Methyl methanesulfonate (MMS)	Aldrich Chemical Co., Inc.	09419LR		>99 %	DMSO
2-Nitrofluorene (2NF)	Aldrich Chemical Co., Inc.	11202TF		>98 %	DMSO
Sodium azide (SA)	Sigma Chemical Company	098H0169	Practical	>99 %	water
Sterigmatocystin (STM)	Sigma Chemical Company	118H4061		>99 %	DMSO

(2) Preparation and storage of positive control solution

Prepare or Store	Store subdivided solutions (Storage temp. -5 to –30°C)

5. Preparation of Test Substance Solution

Solvent used	*Name	*Manufacture	*Lot No.	*Grade	*Purity (%)
	Acetone	Fisher Scientific	970721; 943596; 971014	Certified ACS	
* Stability of test substance in the solvent	Unknown				
* Reason to choose the solvent	Solubility determination				
Method of suspension when test substance is difficult to dissolve	Not applicable				
* Storage time and temp. from preparation to use for test	<30 minutes at ambient temperature				
* Conversion by purity	No				

6. Conditions of Pre-culture

(1) Conditions

Nutrient broth	Name	*Manufacturer	*Lot No.
	Oxoid Nutrient Broth No. 2	Oxoid Ltd.	CH,-B=219916
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		

7. 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

8. Test Results - Judgement of the results

Judgement	Negative
Reason for judgement and referential matters: No positive response was observed with any of the tester strains in the presence and absence of Aroclor-induced rat liver S9 up to 5000 µg/plate.	

Referential matters

The vehicle and positive control values indicate that all tester strains were functioning correctly and were capable of detecting a mutagen. The vehicle controls exhibited the characteristic numbers of spontaneous revertants and the test results are valid.