
# AFRL-RH-WP-TR-2012-0011

**Acute Dermal Irritation Study and**
***Salmonella-Escherichia coli*/**
**Microsome Plate Incorporation Assay of**
**Hydroprocessed Esters and Fatty Acids (HEFA)**
**Bio-Based Jet Fuels**

**David R. Mattie**
**Bioeffects Division**
**Molecular Bioeffects Branch**

**Jonathon M. Hurley**
**WIL Research Laboratories LLC**
**Ashland OH**

**Edward S. Riccio**
**SRI International**
**Biosciences Division**
**Menlo Park CA**

**Teresa R. Sterner**
**Henry M. Jackson Foundation**
**for the Advancement of Military Medicine**
**Wright-Patterson AFB OH**

**January 2013**

**Interim Report for October 2010 to April 2012**



**Air Force Research Laboratory**
**711th Human Performance Wing**
**Human Effectiveness Directorate**
**Bioeffects Division**
**Molecular Bioeffects Branch**
**Wright-Patterson AFB OH 45433**

# NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

The experiments reported were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

AFRL-RH-WP-TR-2013-0011 HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

David R Mattie

David R. Mattie, Work Unit Manager
Molecular Bioeffects Branch

GARRETT D. POLHAMUS, DR-IV, DAF
Chief, Bioeffects Division
Human Effectiveness Directorate
711th Human Performance Wing
Air Force Research Laboratory

This report is published in the interest of scientific and technical information exchange and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 31-Jan-2013 | Interim | Oct 2010 – Apr 2012 |

**4. TITLE AND SUBTITLE**
Acute Dermal Irritation Study and *Salmonella-Escherichia coli*/Microsome Plate Incorporation Assay of Hydroprocessed Esters and Fatty Acids (HEFA) Bio-Based Jet Fuels

**5a. CONTRACT NUMBER**
FA8650-10-2-6062

**5b. GRANT NUMBER**
NA

**5c. PROGRAM ELEMENT NUMBER**
62202F

**6. AUTHOR(S)**
Mattie, David R.*; Hurley, Jonathon M.[1]; Riccio, Edward S.[2]; Sterner, Teresa R.[3];

**5d. PROJECT NUMBER**
OAFW

**5e. TASK NUMBER**
P0

**5f. WORK UNIT NUMBER**
OAFWP002

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
[1]WIL Research Laboratories, LLC, 1407 George Road, Ashland OH 44805-8946
[2]SRI International, Biosciences Division, 333 Ravenswood Ave, Menlo Park CA 94025
[3]HJF, 2729 R St, Bldg 837, WPAFB OH 45433-5707

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
Air Force Materiel Command*
Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Bioeffects Division
Molecular Bioeffects Branch
Wright-Patterson AFB OH 45433-5707

**10. SPONSOR/MONITOR'S ACRONYM(S)**
711 HPW/RHDJ

**11. SPONSORING/MONITORING AGENCY REPORT NUMBER**

**12. DISTRIBUTION AVAILABILITY STATEMENT**
Distribution A: Approved for public release; distribution unlimited.

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The U.S. Air Force is developing alternative fuels to decrease dependence on foreign oil. All new fuels are potentially hazardous to Air Force personnel and require toxicity evaluation. The objective of the dermal irritation study was to determine the irritative potential of three biobased jet fuels, Hydroprocessed Esters and Fatty Acids-Camelina (HEFA-C), HEFA-Tallow (HEFA-T) and HEFA-Animal Fats and Oils (HEFA-F), and to compare the results with the irritative potential of petroleum based JP-8. Exposures consisted of a single occluded or semi-occluded application to the skin of albino rabbits. Dermal irritation scores for all three HEFA fuels and JP-8 indicated that the fuels were slightly irritating to rabbit skin. One exposure (HEFA-F, semi-occluded) was non-irritating. The *Salmonella-Escherichia coli*/microsome plate incorporation assay examined two HEFA fuels, HEFA-C and HEFA-T, for mutagenic activity. HEFA-C and HEFA-T were judged to be non-mutagenic under the test conditions used in this study; therefore, the test substances were determined to be negative in the bacterial reverse mutation assay.

**15. SUBJECT TERMS**
Alternative fuels, Jet Fuel, Dermal, Dermal Irritation, Rabbit

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| U | | | | | David R. Mattie |
| a. REPORT | b. ABSTRACT | c. THIS PAGE | SAR | 118 | 19b. TELEPONE NUMBER (*Include area code*) |
| U | U | U | | | NA |

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI-Std Z39-18

THIS PAGE INTENTIONALLY LEFT BLANK.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

THIS PAGE INTENTIONALLY LEFT BLANK.

## PREFACE

Funding for this project was provided through the Alternative Fuels Certification Office (AFLCMC/WNN). This research was conducted under contract FA8650-10-2-6062 with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF). The program manager for the contract was David R. Mattie, PhD (711 HPW/RHDJ), who was also the technical manager for this project.

The dermal irritation study protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (U.S. EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870. 2500 (1998a) and the Organisation for Economic Co-operation and Development Guidelines for Testing of Chemicals, Section 404 (OECD, 2002).

The *Salmonella-Escherichia coli*/microsome plate incorporation assay testing procedures were consistent with the OPPTS, Health Effects Test Guidelines, 870.5100 (U.S. EPA, 1998b).

Both studies were conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP).

The dermal irritation study was approved by the Air Force Surgeon General's Office of Research Oversight and Compliance (protocol number AFMC-2011-001A) and the WIL Research Laboratories, LLC, Animal Care and Use Committee (protocol number WIL-773002). The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

THIS PAGE INTENTIONALLY LEFT BLANK.

## 1.0 SUMMARY

The U.S. Air Force is developing alternative fuels to decrease dependence on foreign oil. All new fuels are potentially hazardous to Air Force personnel and require toxicity evaluation. The objective of the dermal irritation study was to determine the irritative potential of three biobased jet fuels, Hydroprocessed Esters and Fatty Acids (HEFA)-Camelina (HEFA-C), HEFA-Tallow (HEFA-T) and HEFA-Animal Fats and Oils (HEFA-F), in comparison with the irritative potential of petroleum based JP-8, following a single occluded or semi-occluded exposure to the skin of New Zealand White (NZW) rabbits. Two groups of three male rabbits received a single occluded or semi-occluded exposure to each test substance. Doses (0.5 mL) of each fuel were applied to separate areas of clipped, unabraded skin (four application sites/rabbit). After four hours of exposure, the bandages were removed and the sites washed. Application sites were evaluated in accordance with the method of Draize (1965) and in compliance with U.S. EPA (1998a) at approximately 30 to 60 minutes and 24, 48 and 72 hours after patch removal, and on study days 4, 7 and 14, if irritation persisted.

There were no deaths or remarkable body weight changes noted during the study. Dermal findings during the study consisted of very slight (grade 1) to slight (grade 2) erythema. A score of slightly irritating, as evaluated by the Primary Dermal Irritation Index (PDII) and Descriptive Rating, was determined for the occluded and semi-occluded exposures to all test substances except HEFA-F was found to be non-irritating in the semi-occluded exposure.

The *Salmonella-Escherichia coli*/microsome plate incorporation assay examined two HEFA fuels, HEFA-C and HEFA-T, for mutagenic activity. The assay was performed using the plate incorporation procedure with *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strain WP2 (*uvr*A) in both the presence and absence of a metabolic activation mixture (MA) containing an Aroclor 1254-induced rat-liver S9. The range-finding experiment was conducted for both fuels with strain TA100 over doses of 0.156, 0.313, 0.625, 1.25, 2.5 and 5 µL/plate (100 µL) in the presence and absence of MA containing 5 percent S9. No cytotoxicity was seen at any dose level. The mutagenicity experiments were conducted with all five tester strains at doses of 0.156, 0.313, 0.625, 1.25, 2.5 and 5 µL/plate, in the presence and absence of a metabolic activation system containing 5 percent S9 (first experiment) and 10 percent S9 (second experiment).

Slight increases in a number of revertant colonies were determined to be statistically significant ($p<0.01$); however, revertant colonies were generally less than 2-fold, within the historical range for the strain, not reproducible, and, for the most part, not dose-dependent by regression analysis. Therefore, they were not considered to be a mutagenic response or biologically relevant. Cytotoxicity was not seen under any test condition. HEFA-C and HEFA-T were judged to be non-mutagenic under the test conditions used in this study; therefore, the test substances were determined to be negative in the bacterial reverse mutation assay.

## 2.0 INTRODUCTION

The U.S. Air Force is in the process of developing alternative fuels in order to decrease dependence on foreign oil. Since occupational exposures are potentially hazardous to Air Force personnel, new fuels require evaluation for toxicity. Fischer Tropsch (F-T) fuel was the first alternative jet fuel to be tested and certified for use in the U.S. Air Force fleet. The second class of alternative fuels was initially called hydrotreated renewable jet (HRJ). The HRJ fuels are now referred to as Hydroprocessed Esters and Fatty Acids (HEFA). There are three HEFA fuels being developed and are named based on their starting feedstock: HEFA-C is from camelina oil; HEFA-T is from tallow (rendered beef fat); and HEFA-F is from mixed animal fats and oils. These alternative jet fuels are undergoing toxicological evaluation by the 711Human Performance Wing, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffect Branch (711 HPW/RHDJ). The results of the toxicity studies of the alternative fuels will be compared with the toxicity of traditional petroleum distillate JP-8 jet fuel. Dermal irritation and mutagenic activity are important studies to evaluate the acute toxicity of a chemical or mixture and are part of the required tests for certification of new fuels (DoD, 2010).

JP-8 was previously evaluated in a skin irritation protocol designed by Draize (1965). Results from this rabbit skin irritation test varied: non-irritating (Smith *et al.*, 1981); slightly irritating (Kinkead *et al.*, 1992); non-irritating (Wolfe *et al.*, 1996); and moderately irritating (Hurley *et al.* (2011). JP-8 is commonly combined with anti-icing and anti-corrosion chemicals, known as the JP-8+100 packages. All JP-8+100 packages were non-irritating when tested for skin irritation (Wolfe *et al.*, 1996). Minor differences in how the studies were conducted may have resulted in the different outcomes. It is also possible that the +100 additives prevented the onset of dermal irritation. In operational use, JP-8 appears to cause dermal irritation to humans based on anecdotal reporting received from operational and medical personnel (Chao *et al.*, 2005). Because of the variable results in past JP-8 animal studies and dermal toxicity seen in humans, it is important to test each new fuel for dermal irritation.

Few dermal systemic toxicity tests, aside from the acute irritation tests discussed above, have been performed with JP-8 itself. A good review of the dermal toxicity of petroleum distillates closely related to JP-8 can be found in McDougal and Rogers (2004). One subchronic dermal study of JP-8 was conducted by Baker and coauthors in 1999. Dermal histological changes were investigated in male F344 rats. A daily un-occluded dermal exposure to 0.156 mL JP-8, JP+100 or JP-4 for four weeks was followed by a three-week recovery period. Proliferative, degenerative and inflammatory changes were significantly greater in the fuel-exposed skin versus non-exposed control skin sites on the same animal immediately post-exposure, but fuel treatment results did not differ from each other. Following the recovery period, the dermal histology of all the exposed skin sites had returned to control scores (Baker et al., 1999). The study herein was designed to evaluate the irritation potential of all three HEFA fuels as well as re-evaluate the irritative potential of JP-8, following a single exposure to the skin of New Zealand White (NZW) albino rabbits.

JP-8 was also previously evaluated for mutagenetic activity in the *Salmonella-Escherichia coli*/microsome plate incorporation assay (Brusick and Matheson, 1978). Microbial mutagenicity assays, developed first by Ames *et al.* (1975), are capable of rapidly detecting the

mutagenic activity of a wide range of chemical classes. Many chemicals that elicit a mutagenic response in the *Salmonella* assay have been shown to be potentially mutagenic and carcinogenic to humans and laboratory animals. One advantage of also using the procedure with *E. coli* is that this strain has an A-T base-pair at the critical mutation site and thus is sensitive to some mutagenic chemicals that are not detected by the *Salmonella* strains. Because microbial mutagenicity assays are short-term, sensitive, and reliable tests for assessing mutagenic potential, their use for genotoxic evaluation of chemicals is appropriate. The study herein was designed to evaluate the mutagenic potential of two HEFA fuels (C and T) in a microbial plate incorporation assay.

### 2.1 Objectives

The objective of the dermal irritation study was to determine the irritative potential of three biobased jet fuels, HEFA-C, HEFA-T and HEFA-F, in comparison with the irritative potential of petroleum based JP-8 following a single occluded or semi-occluded exposure to the skin of NZW albino rabbits. This study is intended to provide information on the health hazards likely to arise from a short-term exposure to the test substances by the dermal route.

The protocol (Appendix A) was designed to be in general compliance with the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.2500 (1998a) and the Organisation for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (2002). The study was conducted in compliance with the U.S. EPA Good Laboratory Practices (GLP, 40 CFR Part 792), with the exception that analytical confirmation of the concentration, homogeneity and stability of the fuels was not performed.

The objective of the *Salmonella-Escherichia coli*/microsome plate incorporation assay was to evaluate the ability of two bio-based jet fuels, HEFA-C and HEFA-T, to induce genetic damage as detected by the *Salmonella-E. coli*/microsome assay (Ames test). The protocol can be found in Appendix B. The purpose of this study was to provide data relating to the test substance's health effects, environmental effects, or environmental fate testing regulated by the U.S. EPA. This study, therefore, was conducted in compliance with 40 CFR Part 792 (GLP). Testing procedures were consistent with the OPPTS Health Effects Test Guidelines, 870.5100 (1998b).

## 3.0 METHODS: DERMAL IRRITATION STUDY

### 3.1 Test Substance Identification

The test substances were received from 711 HPW/RHDJ, Wright-Patterson Air Force Base OH. Jet fuels were originally provided by AFRL Fuels Branch (RQPF) and are identified by POSF log book numbers assigned by the Fuels Branch. Test substance identification and physical descriptions are located in Table 1.

**Table 1. Test Substance Identification and Physical Descriptions**

| Fuel | POSF Lot Number | WIL Log Number | Physical Description |
|---|---|---|---|
| JP-8 | POSF 4658 | 8499A | Clear, light yellow liquid |
| HEFA-Camelina | POSF 6152 | 8497A | Clear, colorless liquid |
| HEFA-Tallow | POSF 6308 | 8496A | Clear, colorless liquid |
| HEFA-Animal Fats and Oils | POSF 5469 | 8498A | Clear, colorless liquid |

Purity and stability data were the not the responsibility of WIL Research but was provided by the Air Force. The test substances were stored at room temperature in a flame cabinet and were considered stable under these conditions. A reserve sample of each test substance was collected and stored in the WIL Research Archives.

Prior to use, the original containers of each test substance was inverted to ensure a homogeneous mixture. A vial of each test substance provided by the Air Force was dispensed for dosing.

### 3.2 Test System, Animal Receipt and Acclimation

New Zealand White albino rabbits were used as the test system on this study. This animal model is generally recognized as appropriate for acute dermal irritation studies. The animals were approximately 46 to 52 weeks old at the initiation of dose administration and a minimum of 2 kg body weight.

Male NZW albino rabbits, in good health, utilized for this study were transferred from the WIL Research acute stock colony on 18 January 2011. The rabbits were weighed and uniquely identified by a plastic ear tag displaying the animal number. The rabbits were acclimated to laboratory conditions for a minimum of five days. During this period, each animal was observed twice daily for mortality and changes in general appearance or behavior.

### 3.3 Animal Care

Upon arrival, all animals were housed in individual stainless steel cages elevated above ground containing corncob bedding that was changed at least twice weekly. The animals were maintained by the animal husbandry staff of WIL Research in accordance with standard operating procedures (SOPs). The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

The basal diet used in this study, PMI Nutrition International, LLC (St. Louis MO), Certified High Fiber Rabbit LabDiet® 5325, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research. Municipal water supplying the facility was

analyzed for contaminants according to WIL Research SOPs. The results of the diet and water analyses are maintained at WIL Research. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. The basal diet was provided at approximately 150 g per day while municipal water, delivered by an automatic watering system, was provided *ad libitum* throughout the acclimation period and during the study.

All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain environmental conditions of 66 ± 5 °F (19 ± 3°C) and 50 ± 20 percent, respectively. Room temperature and relative humidity data were monitored continuously and were scheduled for automatic collection on an hourly basis. These data are summarized in Appendix C. Actual mean daily temperatures ranged from 65.2 °F to 65.9 °F (18.4 °C to 18.8 °C) and mean daily relative humidity levels ranged from 30.6 percent to 53.8 percent during the study. Fluorescent lighting provided illumination for a 12-hour light (0600 hours to 1800 hours) and 12-hour dark photoperiod. The light status (on or off) was recorded once every 15 minutes. Air handling units were set to provide a minimum of ten fresh air changes per hour.

### 3.4 Assignment of Animals to Treatment Groups

Animals used in the study were arbitrarily selected from available stock based upon health and body weight. Body weight values ranged from 3174.6 g to 3972.5 g at initiation of dosing.

### 3.5 Test Substance Administration

There were two groups of three rabbits with four unabraded sites per rabbit (Figure 1). The location of the test sites (designated A through D based upon four available site locations on the back of the rabbit) were rotated so that no test substance was applied to the same site within a group of rabbits. The test sites were delineated with four dots made with indelible ink spaced approximately 2.5 centimeters apart arranged in a square.

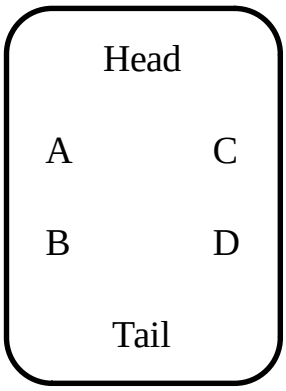



**Figure 1. Location of Rabbit Dermal Test Sites**

Each animal received a single, four-hour, occluded or semi-occluded exposure (Groups 1 and 2, respectively) of each test substance on separate areas of intact, unabraded skin. Table 2 presents the study group assignment. No separate control group was utilized; each animal served as its own control.

**Table 2. Study Group Assignment**

| Group Number | Test Substances | Dose Volume (mL)* | Exposure Method | Number of Animals |
|---|---|---|---|---|
| 1 | JP-8, HEFA-C HEFA-T, HEFA-F | 0.5 | Occluded | 3 |
| 2 | JP-8, HEFA-C HEFA-T, HEFA-F | 0.5 | Semi-occluded | 3 |

Note: *0.5 mL/site, unabraded

On the day prior to dosing, the hair was removed from the backs and flanks of the rabbits using an electric small animal clipper. The clipped area on each animal constituted approximately 20 to 25 percent of the total body surface area. Animals with obvious dermal abnormalities or injuries would have been excluded from the study.

Each 0.5-mL dose was applied to an area of skin approximately 2.5 cm x 2.5 cm under a two-ply gauze patch secured in place with Micropore™ tape (3M, St.Paul MN). For animals in the occluded group, the trunk of the animal was wrapped with plastic wrap. The trunks of animals in both the occluded and semi-occluded group were then wrapped with a gauze binder and secured with Dermiform® tape (Johnson & Johnson, New Brunswick NJ). Plastic restraints (Elizabethan) collars were applied to the animals to prevent ingestion of the test substance and/or bandages. After four hours of exposure, the collars and bandages were removed and each of the sites was wiped with a new disposable paper towel moistened with deionized water. Care was taken not to irritate the skin with the towel.

The selected route of administration for this study was direct application to clipped, unabraded skin (dermal). This route is standard for assessment of local dermal irritative potential. This study was intended to provide information on the health hazards likely to arise from a short-term exposure to the test substances by the dermal route. The experimental design used the procedures and standards required by the current federal and international regulations.

### 3.6 Parameters Evaluated

*3.6.1 Mortality.* The rabbits were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity, including general appearance and behavior. All animals received detailed physical examinations on the day of dosing.

*3.6.2 Body Weights.* Body weights were obtained and recorded on study day 0 (initiation) and at study termination (study day 14).

*3.6.3 Dermal Observations.* The application sites were observed for erythema, edema, and other dermal findings approximately 30 to 60 minutes and 24, 48 and 72 hours after patch removal, and on study days 4, 7 and 14, if irritation persisted. Dermal irritation was graded in accordance with the method of Draize (1965) and in compliance with U.S. EPA (1998a) (see Table 3). The areas of application were clipped free of hair a minimum of one hour prior to scoring, as needed during the study, to facilitate accurate dermal observations.

**Table 3. Evaluation of Dermal Reactions**

| Value | Erythema and Eschar Formation |
|---|---|
| 0 | No erythema |
| 1 | Very slight erythema (barely perceptible, edges of area not well defined) |
| 2 | Slight erythema (pale red in color and edges definable) |
| 3 | Moderate to severe erythema (definite red in color and area well defined) |
| 4 | Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth |
| 4 | Maximum possible erythema score |
| | |
| **Value** | **Edema Formation** |
| 0 | No edema |
| 1 | Very slight edema (barely perceptible, edges of area not well defined) |
| 2 | Slight edema (edges of area well defined by definite raising |
| 3 | Moderate edema (raised approximately 1 mm) |
| 4 | Severe edema (raised more than 1 mm and extending beyond area of exposure) |
| 4 | Maximum possible edema score |
| | |
| 8 | Maximum total possible Primary Irritation Score |

Note: Scoring system from Draize (1965), in compliance with U.S. EPA (1998a)

*3.6.4 Calculation of Primary Dermal Irritation Index.* The Primary Dermal Irritation Index was calculated from scores recorded at 30 to 60 minutes and at 24, 48 and 72 hours after patch removal for the four-hour exposure test sites. The mean scores for erythema and edema were

calculated separately to the nearest tenth and added together. Based on this value, the grading system in Table 4 was used to arrive at the primary dermal irritation descriptive rating.

**Table 4. Descriptive Ratings: Mean Primary Dermal Irritation Index**

| Mean Range of Values | Descriptive Rating |
|---|---|
| 0 | Nonirritating |
| 0.1 - 2.0 | Slightly Irritating |
| 2.1 – 5.0 | Moderately Irritating |
| 5.1 – 8.0 | Severely Irritating |

### 3.7 Termination

After study termination, the rabbits were euthanized by intravenous injection of sodium pentobarbital. The carcasses were discarded without further evaluation.

### 3.8 Data Acquisition and Reporting

The Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) has title to all documentation records, raw data, or other work product generated during the performance of the study. All remaining work product generated by WIL Research, including raw paper data, are retained in the WIL Research Archives as specified in the study protocol. Archive software systems utilized by WIL are detailed in Table 5.

**Table 5. Archive Software Systems**

| Program/System | Description |
|---|---|
| Archive Management System (AMS) | In-house developed application for storage, maintenance, and retrieval of information for archived materials (e.g., lab books, study data, wet tissues, slides, etc.) |
| InSight® Publisher | Electronic publishing system (output is Adobe Acrobat, PDF) |
| Master Schedule | Maintains the master schedule for the company |
| Metasys DDC Electronic Environmental Control System | Controls and monitors animal room environmental conditions |
| Microsoft® Office 2002 and 2007; GraphPad Prism® 2008 | Used in conjunction with the publishing software to generate study reports |
| WIL Metasys | In-house developed system used to record and report animal room environmental conditions |

Reserve samples of the test substances, pertinent electronic storage media, and the original final report are retained in the WIL Research Archives in compliance with regulatory requirements.

## 4.0 RESULTS: DERMAL IRRITATION STUDY

There were no deaths during the study. There were no remarkable body weight changes noted during the study (Table 6).

**Table 6. Individual Body Weights**

| Group | Number | Sex | Initiation (Day 0, g) | Termination (Day 14, g) |
|---|---|---|---|---|
| 0.5 mL/site, unabraded, occluded | 60224 | M | 3454.6 | 3453.2 |
| | 61517 | M | 3202.8 | 3136.0 |
| | 60223 | M | 3972.5 | 3901.6 |
| | | | | |
| 0.5 mL/site, unabraded, semi-occluded | 61523 | M | 3174.6 | 3194.8 |
| | 60201 | M | 3434.0 | 3482.1 |
| | 60222 | M | 3465.9 | 3469.3 |

### 4.1 Dermal Observations

Dermal findings noted during the study consisted of very slight (grade 1) to slight (grade 2) erythema (Table 7). Individual results are found in Appendix D. Irritation was noted for the occluded and semi-occluded exposures to all test substances, with the exception of the semi-occluded exposure to HEFA-F for which no irritation was noted. Very slight edema was limited to a single occluded exposure to JP-8 on study day 7. Very slight erythema persisted through study day 14 for the occluded exposures to JP-8 (two animals) and HEFA-T (one animal), as well as the semi-occluded exposures to JP-8 (two animals).

**Table 7. Dermal Irritation Scores for JP-8 and Three HEFA Biofuels**

| Test Substance | Exposure | PDII | Descriptive Rating |
|---|---|---|---|
| JP-8 | Occluded | 0.8 | Slightly Irritating |
| | Semi-Occluded | 0.8 | Slightly Irritating |
| HEFA-C | Occluded | 0.9 | Slightly Irritating |
| | Semi-Occluded | 0.6 | Slightly Irritating |
| HEFA-T | Occluded | 0.6 | Slightly Irritating |
| | Semi-Occluded | 0.2 | Slightly Irritating |
| HEFA-F | Occluded | 0.3 | Slightly Irritating |
| | Semi-Occluded | 0 | Nonirritating |

### 4.2 Quality Assurance

Study signature pages and quality assurance statements are located in Appendix E.

## 5.0 METHODS: *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY

### 5.1 Design Synopsis

The route of administration was diluted test article (Table 8) added to an agar containing test system. The plate incorporation method is the standard route to administer a test article to the test system for microbial genotoxicity evaluation of chemicals. The protocol and amendments are presented in Appendix B.

## Table 8. Test and Control Substances

| | | |
|---|---|---|
| **Test Substances** | Names | HEFA-Camelina (POSF6152)<br>HEFA-Tallow (POSF6308) |
| | Supplier | Air Force Research Laboratory (Wright-Patterson Air Force Base OH) |
| | Purity | Reported by Sponsor to be greater than 99% |
| | Physical Descriptions | Clear, colorless liquids |
| | Storage Conditions | 15°–26.5°C |
| | Characterization | GLP–compliant characterization was not provided by the Sponsor and therefore does not appear in this report |
| | | |
| **Positive Controls Without Activation** | | |
| **For Strains TA1535 & TA100** | Name | Sodium azide |
| | CAS Number | 26628-22-8 |
| | Manufacturer | Sigma-Aldrich Corp. (St. Louis MO) |
| | Lot Number | mkbf6507v |
| | Physical Description | White powder |
| | Storage Conditions | 16°–24°C |
| | Dose/Plate | 5 µg/50 µl |
| | | |
| **For Strain TA1537** | Name | 9-Aminoacridine hydrochloride hydrate |
| | CAS Number | 52417-22-8 |
| | Manufacturer | Sigma-Aldrich Corp. (St. Louis MO) |
| | Lot Number | 07620td |
| | Physical Description | Yellow powder |
| | Storage Conditions | 16°–24°C |
| | Dose/Plate | 5 µg/50 µl |
| | | |
| **For Strain TA98** | Name | 2-Nitrofluorene |
| | CAS Number | 607-57-8 |
| | Manufacturer | Sigma-Aldrich Corp. (St. Louis MO) |
| | Lot Number | s43858 |
| | Physical Description | Dull, yellow powder |
| | Storage Conditions | 16°–24°C |
| | Dose/Plate | 5 µg/50 µl |
| | | |
| **For Strain WP2 (*uvr*A)** | **Name** | 4-Nitroquinoline N-oxide |
| | **CAS Number** | 56-57-5 |
| | **Manufacturer** | Sigma-Aldrich Corp. (St. Louis MO) |
| | **Lot Number** | 090m1161v |
| | **Physical Description** | Yellow powder |
| | **Storage Conditions** | -19.8° − -14°C |
| | **Dose/Plate** | 2.5 µg/50 µl |
| | | |

**Table 8. Test and Control Substances (continued)**

| | | |
|---|---|---|
| **Positive Control With Activation** | **Name** | 2-Anthramine (2-Aminoanthracene) |
| | **CAS Number** | 613-13-8 |
| | **Manufacturer** | Sigma-Aldrich Corp. (St. Louis MO) |
| | **Lot Number** | STBB1901 |
| | **Physical Description** | Green gold powder |
| | **Storage Conditions** | 16°–24°C |
| | **Dose/Plate** | 2 µg/50 µL (TA98 and TA100), 4 µg/50 µL (TA1535 and TA1537), and 20 µg/50 µL [WP2 (*uvr*A)] in the presence of metabolic activation |
| | **Characterization** | Characterization of each positive control article was obtained from the manufacturer's CofA, which is included in Appendix F. |
| | | |
| **Solvent** | **Name** | Dimethyl sulfoxide (DMSO) |
| | **CAS Number** | 67-68-5 |
| | **Manufacturer** | Mallinckrodt Chemicals (Phillipsburg NJ) |
| | **Lot Number** | J12J03 |
| | **Physical Description** | Clear, colorless liquid |
| | **Storage Conditions** | 15°–26.5°C |
| | **Characterization** | Characterization of the solvent was obtained from the manufacturer's Certificate of Analysis (CofA), which is included in Appendix F. |
| | **Preparation of Dose Formulations** | An aliquot of each test substance was added to DMSO to make a 0.05 mL/mL stock solution for the range-finding experiment. For the mutagenicity experiments, a stock solution of 0.05 mL/mL was prepared for both test substances. Each stock concentration was mixed on a vortex mixer for two times 60 sec each (range finding experiment for mutagenicity), 2 min (1st experiment for mutagenicty), 1 min (2nd experiment for mutagenicty). In each of the experiments, serial dilutions were made from the initial stock solution and vortexed for 30 sec (range finding, 1st experiment, and 2nd experiment for mutagenicity) between dilutions. Dose formulations were prepared at room temperature, under yellow light, and used on the day they were prepared. |
| | **Characterization of Dose Formulations** | Assays to verify the stability, homogeneity, and concentration of each test substance in the vehicle are the responsibility of the Sponsor and are not contained in this final report. |
| | **Disposition** | Unused bulk test substance will be returned to the Sponsor. Unused dose formulations, not reserved for dose concentration analysis, were discarded immediately after use in the test system. |
| | **Test Substance Handling** | The test substance and dose formulations were handled with the use of eye protection, gloves, and a protective smock or laboratory coat. |

## 5.2 Test System

The *Salmonella* tester strains (Table 9) have mutations in the histidine operon, a mutation that leads to a defective lipopolysaccharide coat (*rfa*), and a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (UV)-induced DNA damage (*uvr*B). The *rfa* mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The *uvr*B mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain

TA1535 is reverted to histidine independence by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the drug resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens (McCann *et al.*, 1975). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker for detecting the presence of the plasmid in the cell (Mortelmans and Stocker, 1979). The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens such as ICR-191. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by adding the plasmid pKM101, which makes it more sensitive to some mutagenic agents (Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

**Table 9. Source of Indicator Organisms**

| Species | Strains | Source |
|---|---|---|
| *Salmonella typhimurium* LT2 | TA1535, TA1537, TA98, TA100 | Dr. Bruce Ames, University of California, Berkeley |
| *Escherichia coli* | WP2 (*uvr*A) | National Collection of Industrial and Marine Bacteria (NCIMB) (Aberdeen, Scotland) |

The *E. coli* WP2 (*uvr*A) strain carries a mutation at the tryptophan (*trp*) allele, which is an auxotrophic mutation reverted by base-pair substitution. The strain is deficient in the repair of UV-induced DNA damage (*uvr*A) (Bridges, 1972; Green and Muriel, 1976; Mortelmans and Riccio, 2000) and thus has enhanced sensitivity to some mutagenic agents.

The strains were analyzed for their genetic markers and for the presence of the plasmid whenever experiments were performed. The indicator strains were kept frozen at -80ºC in nutrient broth supplemented with 10 percent sterile glycerol. New frozen stock cultures were made from single colony isolates.

Cultures were inoculated into 50 mL Oxoid Nutrient Broth No. 2 (CM 67) and allowed to sit unshaken for 2 to 4 hours, then gently shaken (125 rpm) for 12 hours at 37 ºC. Plates were labeled with indelible ink to identify the test substance, the strain, the dose level, and the presence or absence of the metabolic activation system.

Metabolic activation was induced by an Aroclor 1254-induced rat liver homogenate preparation (S9) supplied by Molecular Toxicology, Inc. (Boone NC) (range-finding and first mutagenicity experiments: Lot No. 2673, 40.70 mg/mL protein; first and second mutagenicity experiments: Lot No. 2708, 41.6 mg/mL protein). The supplier induces liver enzymes of adult male Sprague-Dawley rats by injecting them with Aroclor 1254 (500 mg/kg) five days before they are euthanized. The S9 consists of 9000 × *g* supernatant of liver homogenized in KCl (1 g wet weight of liver to 3 mL of 0.154 M KCl). Dilutions from each lot of S9, ranging from 0.2 to 10 percent in S9 mix, were tested for their ability to activate benzo(a)pyrene and 2-aminoanthracene to intermediates mutagenic to TA100, prior to product release. The metabolic activation mixture

(Ames *et al.*, 1975; Maron and Ames, 1983) for the experiment(s) consisted of the components and amounts shown in Table 10.

**Table 10. Preparation of Metabolic Activation Mixture for 50 mL Batch**

| Ingredient | 5% S9 Mix (mL) | 10% S9 Mix (mL) |
|---|---|---|
| Rat liver S9 (Aroclor 1254-induced) | 2.5 | 5.0 |
| $MgCl_2$ (0.4 M) and KCl (1.65 M) salts | 1.0 | 1.0 |
| Glucose-6-phosphate (1 M) | 0.25 | 0.25 |
| NADP (0.1 M) | 2.0 | 2.0 |
| Sodium phosphate buffer (0.2 M, pH 7.4) | 25.0 | 25.0 |
| Sterile distilled water | 19.25 | 16.75 |

## 5.3 Experimental Procedure

To a sterile 13 × 100-mm test tube placed in a 43 °C heating block, the following were added:

1. 2 mL of molten top agar
2. 0.1 mL of indicator organisms (about $10^8$ bacteria)
3. appropriate amount of the test substance
4. 0.5 mL of metabolic activation mixture or buffer

This mixture was stirred gently and then poured onto plates containing about 25 mL of minimal glucose agar. For WP2 (*uvr*A), the plates were supplemented with a trace of Oxoid nutrient broth. After the top agar had set, the plates were incubated at approximately 37 °C for about 48 hours. The revertant colonies were counted after the incubation period; however, if the plates could not be immediately evaluated, they were refrigerated at approximately 4 °C for one day until they could be counted.

Concurrent sterility, solvent and positive controls were performed with each experiment. Sterility controls included separately plating out each test substance, metabolic activation mixture, and buffer. Solvent controls were performed for the positive controls and consisted of top agar, bacteria, metabolic activation mixture or buffer, and 50 µL dimethyl sulfoxide (DMSO), the solvent used to dissolve the positive control substances. The solvent control for the test substance, referred to as the zero dose, consisted of top agar, bacteria, metabolic activation mixture or buffer, and DMSO. Positive controls were performed with each strain and consisted of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl of the positive control substance.

## 5.4 Experimental Design

A range-finding experiment was conducted with the test substances to determine a suitable dose range for the mutagenicity experiments. It was performed with *Salmonella* tester strain TA100, in the presence and absence of a metabolic activation mixture (MA) containing 5 percent (volume/volume, v/v) Aroclor 1254-induced rat-liver S9, using three plates per dose level. Dose solutions for the range-finding experiment were achieved by preparing a 0.05 mL/mL (5 µL/plate, 100 µL dosing volume) stock solution in DMSO of each test substance and serially diluting with DMSO to obtain doses of 2.5, 1.25, 0.625, 0.313 and 0.156 µL/plate.

For the mutagenicity experiments, the test substances were assessed in two independent experiments using five tester strains in the presence and absence of metabolic activation, with three plates per dose level. Doses for the mutagenicity experiments with both test substances consisted of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µL/plate and were prepared by serially diluting stock solutions of 0.05 mL/mL in DMSO for each test substance. The test substances were initially tested with and without 5 percent (v/v) S9 in the metabolic activation mix and in the second experiment with 10 percent (v/v) S9.

The highest dose level used in the range-finding experiment was 5 µL/plate, the recommended maximum test concentration. Dose selection for the mutagenicity experiments was made to assess the potential dose-response relationship and contained at least three nontoxic dose levels.

## 5.5 Cytotoxicity Assessment

The test plates were compared with the control plates for their revertant count and for the condition of the background bacterial lawn. Toxicity was estimated by several parameters: a substantial decrease in the number of revertant colonies on the test plates, clearing or absence of the background bacterial lawn growth, or formation of pinpoint non-revertant colonies. Endpoints evaluated included the actual numbers of revertant colonies observed on the plates and the condition of the bacterial lawn growth.

## 5.6 Data Collection

Bias was controlled by collecting data with an automated colony counter when possible. The revertant colonies were counted using an automated colony counter. When accurate counts could not be obtained (e.g., because of precipitation on the plates), the colonies were counted manually using an electric probe colony counter. Data were collected using the Sorcerer Image Analysis System (version 2.2), and the Ames Study Manager (version 1.21), made by Perceptive Instruments (Suffolk, England). Counts from the automated colony counter were compared to manual counts prior to collecting data. A complete system calibration is performed annually.

## 5.7 Data Evaluation

An experiment is considered valid when solvent controls are within 10 percent of historical limits for spontaneous revertants, when positive control mutagens elicit a positive response (5-fold increase over the mean value for the solvent for the respective strain), and when there are at least three nontoxic dose levels (mutagenicity experiments). When experimental plates and sterility control plates indicate gross contamination, the results are not considered valid and the experiment is repeated. In addition, whenever experiments are performed, the strains are analyzed to confirm their genetic markers and the presence of the plasmid. If anomalies exist, the experiment is repeated.

Means and standard deviations were calculated from the individual plate counts. Levene's test (Levene, 1960) was performed to determine if a significant difference exists among treatment variances. Treatments were compared with controls using a one-tailed Dunnett's *t*-test (Dunnett, 1980) and within-levels pooled variance. Evaluation of dose-relatedness for all treatments was made by regression analysis (Draper and Smith, 1981) of revertant counts versus the log of the concentrations (to allow inclusion of the zero dose, one was added to the dose before calculating the log). The significance of the regression was tested using a *t*-statistic. The statistical analyses were performed using the SAS analysis system (SAS Institute Inc., Cary NC); the data were read into the SAS program version 9.1 and then the statistical analysis was run on version 6.12.

The following criteria were used as guidelines for the interpretation of the data; however, the conclusions of the study were based upon evaluation and interpretation of the data.

- **Positive.** A test substance is considered a mutagen when a reproducible and statistically significant ($p < 0.01$) increase is observed at one or more dose levels. A statistically significant ($p < 0.01$) dose-related increase in the number of revertants is also considered a positive response.
- **Negative.** A test substance is considered a nonmutagen when the values for the dose levels are not reproducible or significant or when there is no statistically significant dose-related increase in the number of revertants.
- **Inconclusive.** When a test substance cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive.

## 5.8 Regulatory Compliance

This study was conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP), with the exception that the characterization of the test substances (identity, purity, and stability) and the supporting analytical chemistry of the dose formulations were not provided to the testing facility.

The protocol was amended on 14 February 2011 (Amendment No. 1) to specify the dose levels for the first experiment for mutagenicity, and on 11 March 2011 (Amendment No. 2) to specify the dose levels and S9 concentration to be used in the second experiment for mutagenicity.

All raw data, the original protocol and final report, relevant documents and records specific to this study will be stored at SRI International, 333 Ravenswood Avenue, Menlo Park CA 94025. All records will be maintained for at least 10 years. At the end of the retention period, HJF will be contacted regarding further disposition of these records. Wet specimens (e.g., colonies in agar) and samples of the control articles are not required to be retained.

## 6.0 RESULTS: *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY

The presence of the appropriate genetic characteristics was verified for the strains used in this study. The results of the controls were acceptable for all experiments (see historical values in Appendix H) as well as the results of the sterility controls (metabolic activation mix, buffer and a dilution of the test substance). There were an adequate number of nontoxic dose levels in the mutagenicity experiments to evaluate the test substance. Therefore, using these criteria, the assay was considered valid.

### 6.1 Range-Finding Experiment

The range-finding experiment was performed with the two test substances, HEFA-C and HEFA-T, using strain TA100 at doses representing 0.156, 0.313, 0.625, 1.25, 2.5, and 5 μL/plate (100 μL dosing volume) in the presence and absence of a MA system containing 5 percent S9. The dose formulations at 5 μL per plate for both of the test substances appeared to be hazy; no precipitate was seen when they were added to the test system or on the plates when they were counted. Individual and mean plate counts are presented in Appendix G. No dose-related increase in the number of revertant colonies was seen with either test substance. Cytotoxicity was not observed under any test condition.

### 6.2 Mutagenicity Experiments

The first experiment for mutagenicity was conducted with HEFA-C and HEFA-T using all five tester strains at doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 μL/plate in the presence and absence of MA containing 5 percent S9. The dose formulations at 5 μL/plate again appeared to be hazy. The statistical analyses for the experiment and the individual and mean plate counts are presented in Appendix G. No statistically significant increase in the number of revertant colonies was observed with either test substance, except for slight increases seen with HEFA-C at 0.313 μL/plate with TA1537 in the absence of MA, with HEFA-T at 2.5 μL/plate with TA1537 in the absence of MA, and HEFA-T at 0.313 μL/plate with TA1535 in the presence of MA which were statistically significant ($p < 0.01$) by Dunnett's test. Because these increases were so slight, within the historical range for the strains and not dose-dependent, they were not considered to be a mutagenic response or biologically relevant. Cytotoxicity was not evident under the test conditions.

The second experiment for mutagenicity was conducted with all five tester strains over the same range of doses in the presence and absence of MA containing 10 percent S9. The dose formulations at 5 μL per plate appeared to be hazy. The statistical analyses for the experiment and the individual and mean plate counts are presented in Appendix G. Slight increases in the number of revertant colonies were seen with HEFA-C at 0.313 μL/plate with TA98 in the absence of MA and TA100 at 0.156 μL/plate in the absence of MA. These increases were considered statistically significant ($p < 0.01$) by Dunnett's test; however, because they were so slight, within the historical range for the strain, not reproducible, and not dose-dependent, they were not considered to be a mutagenic response or biologically relevant. Dunnett's test found the slight increases in the number of revertant colonies seen with HEFA-T and TA100 at 0.156 μL/plate in the absence of MA and at doses of 0.156 to 5 μL/plate in the presence of MA to be statistically significant ($p < 0.01$). The increases seen over doses of 0.156 to 5 μL/plate with MA were considered to be statistically significant ($p < 0.01$) by regression analysis. Because the increases observed with HEFA-T were so slight, within the historical range for the strain, and not reproducible (in the absence of MA) they were not considered to be a mutagenic response or biologically relevant. No other statistically significant increases in the number of revertant colonies were observed. No signs of cytotoxicity were seen with either test substance.

## 7.0 DISCUSSION AND CONCLUSIONS

The Primary Dermal Irritation Index (PDII) and Descriptive Rating for occluded exposures to the skin of New Zealand White rabbits resulted in a slightly irritating description for JP-8 and all three HEFA fuels. For semi-occluded exposures, JP-8, HEFA-C and HEFA-T resulted in slightly irritating responses, while HEFA-F was non-irritating.

JP-8 was found to be slightly irritating in a previous study by Kinkead *et al*. (1992). In a more recent study by Hurley *et al*. (2011), JP-8 was also reported as slightly irritating under semi-occluded exposure. However, the occluded exposure resulted in moderate irritation, the highest descriptive rating to date. In the same study, F-T was also slightly irritating under semi-occluded exposure and moderately irritating under occluded exposure. Compared to JP-8 and F-T jet fuels, the HEFA jet fuels appear to be less irritating.

HEFA-C and HEFA-T were judged to be nonmutagenic under the test conditions used in this study; therefore, the test substances were determined to be negative in the bacterial reverse mutation assay.

Brusick and Matheson (1978) demonstrated that JP-8 was not mutagenic using the *Salmonella-Escherichia coli*/microsome plate incorporation assay (Ames test), which was a relatively new. More recently, JP-8 was re-tested for mutagenicity when F-T jet fuels were examined for mutagenic effects. Both JP-8 and F-T jet fuels were negative in the bacterial reverse mutation assay (Mattie *et al*., 2011; Riccio *et al*., 2010). To date, each alternative jet fuel tested for mutagenic potential has been shown to be nonmutagenic. Each time petroleum-derived JP-8 has been tested for mutagenicity, the result has been nonmutagenic.

## 8.0 REFERENCES


Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31: 347-364.

Baker, W., Miller, T., Dodd, D., and McDougal, J. 1999. Repeated dose skin irritation study on jet fuels - a histopathology study. Operational Toxicology Branch, Wright-Patterson AFB, OH. AFRL-HE-WP-TR-1999-0022.

Bridges, B. A. 1972. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. 21: 413-416.

Brusick, D. J. and Matheson, D. W. 1978. Mutagen and oncogen study on JP-8. Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH. AMRL-TR-78-20.

Chao, Y. C., Gibson, R. L., and Nylander-French, L. A. 2005. Dermal Exposure to Jet Fuel (JP-8) in U.S. Air Force Personnel. Ann. Occup. Hyg. 49:639-645.

DoD. 2010. Department of Defense Handbook: Aerospace Fuels Certification. Aeronautical Systems Center, Wright-Patterson AFB, OH. MIL-HDBK-510-1A (USAF).

Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics – Dermal Toxicity. Association of Food and Drug Officials of the United States, Topeka, KS. pp. 49-52.

Draper, N. R., and H. Smith, Jr. 1981. Applied Regression Analysis, 2nd ed. John Wiley & Sons, Inc., New York.

Dunnett, C. W. 1980. Pairwise multiple comparisons in the homogeneous variance unequal sample size case. J. Am. Stat. Assoc. 75: 372.

Green, M.H.L., and W. J. Muriel. 1976. Mutagen testing using $trp^+$ reversion in Escherichia coli. Mutat. Res. 38: 3-32.

Hurley, J. M., Wagner, D., Sterner, T. R., and Mattie, D. R. 2011. Acute Dermal Irritation Study of JP-8 and S-8 in New Zealand White Rabbits. Air Force Research Laboratory, Applied Biotechnology Branch, Wright-Patterson AFB, OH. AFRL-RH-WP-TR-2011-0054, ADA546698.

Kinkead, E. R., Salins, S. A., and Wolfe, R. E. 1992. Acute irritation and sensitization potential of JP-8 jet fuel. Acute Toxicity Data. 11:700.

Levene, H. 1960. Robust tests for equality of variance. In Contributions to Probability and Statistics, I. Olkin, Ed. Stanford University Press, Stanford, CA, pp. 278-292.

Maron, D. M., and B. N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 113: 173-215.

Mattie, D. R., Sterner, T. R., Oppong-Nketiah, M., Becker, T., Wallner, B., Riedel, W., Lutterbach, A., and Wagner, D. J. 2011. F-T Jet Fuel Reverse Mutation Assay and Chromosome Aberration Test. Air Force Research Laboratory, 711 Human Performance Wing, Applied Biotechnology Branch, Wright-Patterson AFB, OH. AFRL-RH-WP-TR-2011-0010, ADA536685.

McCann, J., N. E. Spingarn, J. Kobori, and B. N. Ames. 1975. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Natl. Acad. Sci. USA 72: 979-983.

McDougal, J. N. and Rogers, J. V. 2004. Local and systemic toxicity of JP-8 from cutaneous exposures. Toxicol. Lett. 149:301-308.

Mortelmans, K. E., and B.A.D. Stocker. 1979. Segregation of the mutator property of plasmid

R46 from its ultraviolet-protecting property. Mol. Gen. Genet. 167: 317-327.
Mortelmans, K., and Riccio, E. S. 2000. The bacterial tryptophan reverse mutation assay with *Escherichia coli* WP2. Mutat Res. 455:61-69.
Mortelmans, K., and Zeiger, E. 2000. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 455:29-60.
NRC. 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C.: Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.
OECD. 2002. OECD Guideline for the Testing of Chemicals. Guideline 404 Acute Dermal Irritation/Corrosion. Organisation for Economic Co-operation and Development, Paris.
Riccio, E. S., Green, C. E., and Mattie, D. R. 2010. Evaluation of Five Jet Fuels in the Salmonella-Escherichia coli / Microsome Plate Incorporation Assay. Air Force Research Laboratory, 711 Human Performance Wing, Applied Biotechnology Branch, Wright-Patterson AFB, OH. AFRL-RH-WP-TR-2010-0138, ADA536581.
Smith, L. H., Haschek, W. M., and Witschi, H. 1981. Acute toxicity of selected crude and refined shale oil- and petroleum-derived substances. In: Health effects investigation of oil shale development. W. H. Griest, M. R. Guerin, and D. L. Coffin, eds. Ann Arbor, MI: Ann Arbor Science Publishers, Inc. Ch. 11. pp. 141-160.
U.S. EPA. 1998a. Health Effects Test Guidelines: OPPTS 870.2500 Acute Dermal Irritation. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 712-C-98-196.
U.S. EPA. 1998b. Health Effects Test Guidelines: OPPTS 870.5100 Bacterial Reverse Mutation Test. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 712-C-98-247.
Wolfe, R. E., Kinkead, E. R., Feldmann, M. L., Leahy, H. F., Jederberg, W. W., Mattie, D. R., and Still, K. R. 1996. Acute toxicity evaluation of JP-8 jet fuel and JP-8 jet fuel containing additives. Armstrong Laboratory, Wright-Patterson AFB, OH. AL/OE-TR-1996-0136.

# APPENDIX A. DERMAL IRRITATION STUDY PROTOCOL



**PROTOCOL**

**ACUTE DERMAL IRRITATION STUDY OF
HRJ CAMELINA, HRJ TALLOW AND HRJ ANIMAL FATS
AND OILS BIO-BASED JET FUELS IN NEW ZEALAND WHITE RABBITS**

**Submitted To:**

**The Henry M. Jackson Foundation
for the Advancement of Military Medicine**
1401 Rockville Pike, Suite 600
Rockville, MD 20852

**WIL Research Laboratories, LLC**
1407 George Road
Ashland, OH 44805-8946

## 1 OBJECTIVE:

To determine the irritative potential of the test substances following a single exposure to the skin of albino rabbits.

This protocol has been designed and the study will be conducted in general compliance with the following guidelines:

Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substance (OPPTS) guideline 870.2500 (1998).

Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (2002).

The European Union (EU) Guideline in the Official Journal of the European Communities [92/69, Annex V, B4 (1992)].

The study will be conducted in compliance with the U.S. EPA Good Laboratory Practices (40 CFR Part 792), with the exception that analytical confirmation of the concentration, homogeneity and stability of the dosing mixture (if prepared) will not be performed.

## 2 PERSONNEL INVOLVED IN THE STUDY:

### 2.1 Sponsor Representative:

David R. Mattie, PhD, DABT
711 HPW/RHPB
2729 R Street, Bldg 837
Wright-Patterson AFB, OH 45433-5707
Phone: (937) 904-9569
E-mail: David.Mattie@WPAFB.AF.MIL

### 2.2 WIL Study Director:

Jonathan M. Hurley, BS
Project Specialist, General Toxicology
Phone: (419) 289-8700
Fax: (419) 289-3650
E-mail: jhurley@wilresearch.com

## 2.3 WIL Departmental Responsibilities:

Teresa D. Morris, BS
Senior Toxicologist, Toxicology
Emergency Contact
Phone: (419) 289-8700
E-mail: tmorris@wilresearch.com

Mark D. Nemec, BS, DABT
President and Chief Operating Officer

Jozef J.W.M. Mertens, PhD, DABT
Senior Director, General Toxicology

Ronald E. Wilson, BS
Director, Informational Systems

Bennett J. Varsho, MPH, DABT
Director, Operations

Sally A. Keets, AS
Senior Operations Manager, Vivarium

Erica L. Lashley, BS, LAT
Operations Manager, Toxicology

Theresa M. Rafeld, CPhT
Group Manager, Formulations Laboratory

Carol A. Kopp, BS, LAT
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Gwendalyn M. Maginnis, DVM
Attending Veterinarian

Robert A. Wally, BS
Manager, Reporting
and Regulatory Technical Services

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

## 3 STUDY SCHEDULE:

| | |
|---|---|
| Proposed Experimental Start Date: | January 18, 2011 |
| Proposed Experimental Termination Date: | February 1, 2011 |
| Proposed Audited Draft Report Date: | March 15, 2011 |

## 4 TEST SUBSTANCES:

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Information on composition and method of synthesis will be held by the Sponsor.

### 4.1 Test Substance #1 Identification / Lot Number:

JP-8 / POSF4658

### 4.2 Test Substance #2 Identification / Lot Number:

HRJ Camelina/ (UOP) - POSF 6152

### 4.3 Test Substance #3 Identification / Lot Number:

HRJ Tallow/ (UOP) - POSF 6308

### 4.4 Test Substance #4 Identification / Lot Number:

HRJ Animal Fats and Oils/ (Syntroleum) - POSF 5469

### 4.5 Purity:

Responsibility of the Sponsor

### 4.6 Stability:

Considered to be stable for years when properly stored.

### 4.7 Physical Descriptions:

To be documented by WIL Research Laboratories, LLC

### 4.8 Storage Conditions:

Store at room temperature. Keep containers closed tightly. Use and store these materials in cool, dry, well-ventilated areas away from heat, direct sunlight, hot metal surfaces and all sources of ignition.

### 4.9 Personnel Safety:

At minimum, appropriate gloves, eye protection and long sleeves (lab coat) are to be worn during dose administration. Refer to Material Safety Data Sheets for complete available information.

### 4.10 Retention Samples:

Retention samples of the test substances (as received) will be collected in accordance with WIL Research Laboratories, LLC SOP No. T2-001.

### 4.11 Unused Test Substances:

Unused portions of the test substances will be returned following the issuance of the final study report to the contact below.

David R. Mattie, PhD, DABT
711 HPW/RHPB
2729 R Street, Bldg 837
Wright-Patterson AFB, OH 45433-5707
Phone: (937) 904-9569
E-mail: David.Mattie@WPAFB.AF.MIL

## 5 TEST SYSTEM:

### 5.1 Species:

Albino rabbit (*Oryctolagus cuniculus)*

### 5.2 Breed:

New Zealand White

### 5.3 Source:

Covance Research Products, Inc. (USDA License # 23-A-0180)
(Documentation of the specific breeding facility will be maintained in the study records and included in the final report.)

**5.4 Number on Study:**

Six animals from the acute stock colony

**5.5 Sex:**

Males and/or females (females will be nulliparous and nonpregnant)

**5.6 Body Weight Range:**

2.0 kg or greater

**5.7 Approximate Age:**

Young adult, at least 12 weeks old at initiation of dosing

**5.8 Identification System:**

Each animal will be uniquely identified by a plastic eartag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, group and study number.

**5.9 Justification for Selection:**

This species and breed is generally recognized as appropriate for acute dermal irritation studies. The number of animals selected is the minimum required to satisfy regulatory guidelines. The experimental design uses the procedures and standards required by the current federal and international regulations.

## 6 SPECIFIC MAINTENANCE SCHEDULE:

**6.1 Animal Housing:**

The animals will be housed individually in stainless steel cages in an environmentally controlled room. Animals will be housed in clean cages elevated above ground corncob bedding or other suitable material that will be changed at least twice each week. Animals will be changed out into clean cages approximately every two weeks. The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

**6.2 Environmental Conditions:**

Controls will be set to maintain the temperature at 66 ± 5°F (19 ± 3°C) and the relative humidity at 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for

automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

### 6.3 Drinking Water:

Municipal water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures (SOPs). Municipal water supplying the laboratory is analyzed for contaminants according to SOPs to ascertain that none are present at concentrations that would be expected to affect the outcome of the study and the results are maintained on file.

### 6.4 Basal Diet:

PMI Nutrition International, LLC Certified High Fiber Rabbit LabDiet® 5325 will be offered at approximately 150 g/day during the study. The amount of feed provided will be an estimate and will not be documented. Standard Operating Procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Analyses are performed and provided by the manufacturer and the results are maintained on file.

## 7 EXPERIMENTAL DESIGN:

### 7.1 Animal Receipt and Acclimation:

Each animal was/will be inspected by a qualified technician upon receipt into the acute stock colony. Animals judged to be in good health and suitable as test animals were/will be acclimated to laboratory conditions for a minimum of five days. All animals were/will be weighed initially and permanently identified. During the acclimation period, each animal will be observed twice daily for changes in general appearance and behavior.

All relevant records and data collected during the acclimation period for animals used on this study will be maintained on file.

### 7.2 Veterinary Care:

Animals will be monitored by the technical staff for any condition requiring possible veterinary care. If any such condition is identified, a staff veterinarian

will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of the protocol.

### 7.3 Route and Rationale of Test Substance Administration:

The route of administration will be dermal (clipped, intact skin) in order to evaluate the dermal irritation potential of the test substances. This study is intended to provide information on the health hazards likely to arise from a short-term exposure to the test substances by the dermal route.

### 7.4 Organization of Treatment Groups:

Following the acclimation period, animals will be arbitrarily selected from available stock based upon health and body weight and assigned to 2 groups of 3 rabbits/group as shown below. No separate control group will be utilized; each animal will serve as its own control. The skin of all test sites will be left intact (unabraded).

| Group Number | Test Substances* | Dose Volume (mL/Test Substance) | Exposure Method | Number of Animals |
|---|---|---|---|---|
| 1 | #1, #2, #3, #4 | 0.5 | Occluded | 3 |
| 2 | #1, #2, #3, #4 | 0.5 | Semi-occluded | 3 |

*#1 = JP-8; #2 = HRJ Camelina; #3 = HRJ Tallow; #4 = HRJ Animal Fats and Oils

### 7.5 Test Material Preparation:

The test substances will be administered undiluted as received at a dosage of 0.5 mL. The pH will be determined and recorded.

### 7.6 Animal Preparation:

On the day prior to dermal applications, the back and flanks of each animal will be clipped free of hair with a small animal clipper. The clipped area on each animal will constitute approximately 20-25% of the total body surface area (actual size of area will not be recorded). Animals with dermal abnormalities or injuries will be excluded.

### 7.7 Method of Administration:

Four sites located lateral to the midline of the back will be selected on each rabbit. The location of the test sites (designated A-D based upon four available site locations on the back of the rabbit) will be rotated so that no test substance is applied to the same site within a group of rabbits. The test sites will be delineated with four dots made with indelible ink spaced approximately

2.5 centimeters apart arranged in a square. All animals will receive a single application of four test substances.

Each test site will be immediately covered with a two ply, 2.5-cm square gauze patch. The patch will be secured in place with surgical porous tape. For animals in the occluded exposure groups the trunk of the animal will be wrapped with plastic wrap to occlude the test site. The trunk of animals in both the occluded and semi-occluded groups will then be wrapped with gauze bandaging that will be secured with several wrappings of non-irritating tape. Elizabethan collars will be applied to each animal during the exposure period to prevent ingestion of the test substance and/or wrappings.

After the four hours of exposure, the bandages will be removed and residual test substance cleansed from the application sites using clean, disposable paper towels moistened with deionized water (as thoroughly as possible without irritating the skin). The same towel will not be used on more than one site.

## 8 OBSERVATIONS:

### 8.1 Viability and Clinical Observations:

All animals will be observed for mortality/moribundity twice daily (morning and afternoon) for the duration of the study. Moribund animals will be removed from study and euthanized by intravenous injection of sodium pentobarbital. All animals will receive a detailed physical examination on the day of dosing.

### 8.2 Dermal Observations:

Approximately 30-60 minutes after test substance removal, each test site will be examined and the degree of erythema and edema recorded according to the Draize technique (Appendix A). The presence of any other dermal findings will also be recorded. Additional examinations will be performed at approximately 24, 48 and 72 hours after patch removal. If no irritation is present at the 72-hour observation, the study may be terminated.

If irritation is present at the end of 72 hours, additional observations will be performed on days 4, 7 and 14, or until irritation subsides. The study need not normally exceed 14 days after application unless specifically requested and authorized by the Sponsor. Individual animals will be terminated if no irritation is present at the 72-hour or any subsequent observation. At the request of the Sponsor, observations may be terminated prior to 14 days and/or resolution of irritation.

The areas of application will be clipped free of hair a minimum of one hour before scoring, as needed during the study, to facilitate accurate dermal observations.

### 8.3 Body Weights:

The body weight of each animal will be determined on study day 0 and at termination.

### 8.4 Gross Pathology:

All animals will be euthanized by intravenous injection of sodium pentobarbital. A gross necropsy examination on major organ systems of the thoracic and visceral cavities will be conducted on all animals found dead or euthanized *in extremis*. Animals euthanized following study termination will be discarded without further examination.

## 9 CALCULATION OF THE PRIMARY DERMAL IRRITATION INDEX:

The Primary Dermal Irritation Index will be calculated from the scores recorded at 30-60 minutes, 24, 48 and 72 hours (after patch removal). The mean scores for erythema and edema will be calculated separately to the nearest tenth and added together. Based on this value, the grading system in Appendix A will be used to arrive at a primary dermal irritation descriptive rating for each test substance for the occluded and unoccluded method of exposure.

## 10 REPORT:

The final report will include, but will not necessarily be limited to, the following: compliance statement, summary, objective, test substance identification and receipt information, methods, observations, mortality, body weights, individual and summarized dermal scores/findings, classification of the test substances based on their dermal irritation properties, results and discussion, key personnel, a signed QAU statement and protocol deviation(s), if any.

WIL Research Laboratories will submit one electronic copy (PDF with an MS Word copy of the report text for editing and comments) of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two-month time frame following submission. Within one month following receipt of the Sponsor's comments, WIL shall provide a revised draft report that incorporates the Sponsor's reasonable revisions and suggestions. One revision will be permitted as part of the cost of the study; additional changes or revisions may be made, at extra cost. WIL will submit the final report within two weeks of receiving authorization from the Sponsor. If the Sponsor's comments and/or authorization to

finalize the report have not been received at WIL within one year following submission of the draft report, WIL may elect to finalize the report following appropriate written notification to the Sponsor. Two electronic copies (PDF) of the final report on CD-R will be provided. Requests for additional paper copies of the final report may result in additional charges.

## 11 RECORDS TO BE MAINTAINED:

All original raw data records (as defined by the applicable GLPs and WIL SOPs) generated by WIL Research Laboratories, LLC will be collected and maintained by WIL Research Laboratories, LLC.

## 12 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, specimens, or other work product generated during the performance of the study. All work product including raw paper data, pertinent electronic storage media and specimens will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

## 13 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with EPA Good Laboratory Practices and adherence to the protocol and to WIL SOPs. The raw data and draft report will be audited by the WIL Quality Assurance Unit to assure that the final report accurately describes the conduct and the findings of the study.

## 14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the

protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

## 15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that, for the study described in this protocol, there are no acceptable non-animal alternatives and the study does not unnecessarily duplicate previous experiments.

- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory SOPs.

- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

## 16 PROTOCOL APPROVAL:

Sponsor approval received by the Study Director via email on November 15, 2010.

**The Henry M. Jackson Foundation for the Advancement of Military Medicine**

David R. Mattie — 1/18/11

David R. Mattie, PhD, DABT
Sponsor Representative

Date

**WIL Research Laboratories, LLC**

1/14/11

Jonathan M. Hurley, BS
Study Director

Date

## APPENDIX A

SCORING CRITERIA FOR DERMAL REACTIONS*

| Value | Erythema and Eschar Formation |
|---|---|
| 0 | No erythema |
| 1 | Very slight erythema (barely perceptible, edges of area not well defined) |
| 2 | Slight erythema (pale red in color and edges definable) |
| 3 | Moderate to severe erythema (definite red in color and area well defined) |
| 4 | Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth) |
| 4 | Maximum possible erythema score |

| | Edema Formation |
|---|---|
| 0 | No edema |
| 1 | Very slight edema (barely perceptible, edges of area not well defined) |
| 2 | Slight edema (edges of area well defined by definite raising) |
| 3 | Moderate edema (raised approximately 1 mm) |
| 4 | Severe edema (raised more than 1 mm and extending beyond area of exposure) |
| 4 | Maximum possible edema score |
| 8 | Maximum total possible Primary Irritation Score |

DESCRIPTIVE RATINGS

Mean Primary Dermal Irritation Index

| Range of Values | Descriptive Rating |
|---|---|
| 0 | Nonirritating |
| 0.1 - 2.0 | Slightly Irritating |
| 2.1 - 5.0 | Moderately Irritating |
| 5.1 - 8.0 | Severely Irritating |

*Draize, J.H., 1965. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas and the EPA-OPPTS Health Effects Test Guidelines (1998).

## APPENDIX B. *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY PROTOCOL AND AMENDMENTS

**Evaluation of Two HRJ Fuels in the *Salmonella-Escherichia coli*/Microsome Plate Incorporation Assay**

| | | |
|---|---|---|
| I. | **SRI STUDY NUMBER:** | G371-11 |
| II. | **SPONSOR:** | Henry M. Jackson Foundation for the Advancement of Military Medicine<br>1401 Rockville Pike, Suite 600<br>Rockville, MD 20852 |
| | **Contract:** | Subaward 699531, Prime Award FA8650-10-2-6A02 |
| | **Sponsor's Representative:** | David R. Mattie, PhD, DABT<br>Chief, Nano/Toxicology Section<br>711 HPW/RHPBA<br>2729 R Street, Bldg 837<br>Wright-Patterson Air Force Base, OH 45433-5707<br>Phone: 937.904.9569<br>Fax: 937.255.1474<br>E-mail: david.mattie@wpafb.af.mil |
| III. | **TESTING FACILITY:** | SRI International<br>Biosciences Division<br>333 Ravenswood Avenue<br>Menlo Park, CA 94025-3493 |
| | **Study Director:** | Edward S. Riccio, BS<br>Phone: 650.859.4032<br>Fax: 650.859.2889<br>E-mail: edward.riccio@sri.com |

**Proposed Experimental Start Date:** February 8, 2011
**Proposed Experimental Termination Date:** March 15, 2011

**IV. APPROVALS:**

David R. Mattie — 31 Jan 11
David R. Mattie, PhD, DABT — Date
Sponsor's Authorized Representative

Edward S. Riccio — 1/31/2011
Edward S. Riccio, BS — Date
SRI Study Director

**REVIEWED BY:**

Thomas Bregante — 1/31/2011
Thomas Bregante, BS — Date
SRI Quality Assurance Unit

**Evaluation of Two HRJ Fuels in the *Salmonella-Escherichia coli*/ Microsome Plate Incorporation Assay**
**SRI Study No. G371-11**

## V. OBJECTIVE AND PURPOSE OF STUDY

The objective of this study is to evaluate the ability of two bio-based hydro renewable jet fuels to induce genetic damage as detected by the *Salmonella-E. coli*/microsome assay.

The purpose of this study is to provide data relating to the test substance's health effects, environmental effects, or environmental fate testing regulated by the U.S. Environmental Protection Agency (EPA). This study, therefore, will be conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP). Testing procedures will be consistent with the Office of Prevention, Pesticides and Toxic Substances (OPPTS), Health Effects Test Guidelines, 870.5100.

## VI. MATERIALS AND METHODS

### A. Experimental Design

**Route of Administration:** Dissolved/diluted test article added to agar containing test system.

**Reason for Route:** Standard route to administer test article to test system for genotoxic evaluation of chemicals.

**Design.** A range-finding experiment will be conducted with the test substances to determine a suitable dose range for the mutagenicity experiments. The range-finding experiment will be performed with *Salmonella* tester strain TA100, in presence and absence of a rat-liver metabolic activation system (S9), using three plates per dose level, over a wide range of doses. For the mutagenicity experiments, the test substances will be assessed in two experiments using five tester strains in the presence and in the absence of metabolic activation, with three plates per dose level over at least five dose levels (to be added by protocol amendment). The test substances will initially be tested with 5% (v/v) S9 in the S9 mix. If no clear dose-related increase in the number of revertant colonies is observed with a test substance, a 10% (v/v) S9 mix will be used in the repeat experiment. If a mutagenic response is obtained with a test substance in the initial experiment with the 5% S9 mix, the assay will be repeated under the same conditions.

**Justification of Dose Levels Selected.** The highest dose level to be used in the range-finding experiment will be based on solubility or a dose representing 5 µl/plate. Dose selection for the mutagenicity experiments will be made to (1) assess a potential dose-response relationship, (2) include at least one dose that exhibits toxicity, or if a toxic level cannot be achieved, (3) contain a high dose of

5 µl/plate, the recommended maximum test concentration for a soluble noncytotoxic test substance.

**Cytotoxicity Assessment.** The test plates will be compared with the control plates for their revertant count and for the condition of the background bacterial lawn. Toxicity is estimated by several parameters: a substantial decrease in the number of revertant colonies on the test plates, clearing or absence of the background bacterial lawn growth, or formation of pinpoint nonrevertant colonies.

**Endpoints Evaluated**. The actual numbers of revertant colonies observed on the plates and the condition of the bacterial lawn growth.

## B. Test and Control Substances

### 1. Test Substances:

| | |
|---|---|
| **Names / Lot Nos.:** | 1) HRJ Camelina (UOP) / POSF6152<br>2) HRJ Tallow (UOP) / POSF6308 |
| **Supplier:** | Air Force Research Laboratory |
| **Purity:** | Reported by Sponsor to be greater than 99% |
| **Physical Descriptions:** | To be specified in the final report |
| **Storage Conditions:** | Store at room temperature, 15° to 30°C. Keep containers closed tightly. Use and store these materials in cool, dry, well-ventilated areas away from heat, direct sunlight, hot metal surfaces and all sources of ignition. |
| **Characterization of Test Substances:** | Characterization, identity, purity, and stability of the test substances will be the responsibility of the Sponsor and this information will not be contained in the final report. |

**Solvent**

| | |
|---|---|
| **Name:** | Dimethyl sulfoxide (DMSO) |
| **CAS No.:** | 67-68-5 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |
| **Physical Description:** | Clear, colorless liquid |
| **Storage Conditions:** | Room temperature, 15° to 30°C |

| | |
|---|---|
| **Characterization of Solvent:** | Characterization of the solvent will be obtained from the manufacturer's Certificate of Analysis. |
| **Preparation of Dose Formulations:** | An aliquot of the test substance will be prepared in the solvent at a maximum concentration of 0.1 ml/ml. If the test substance is not soluble, it will be gradually diluted until solubility is achieved. Once the stock concentration is prepared, serial dilution will be made from the initial stock. All dose formulations will be prepared at room temperature and mixed thoroughly on a mixing device (at least 5 sec) to ensure homogeneity and adequate solubility. Unless otherwise specified, dose formulations not used on the day of preparation will be stored refrigerated and protected from light. They will be brought to room temperature prior to exposure to the test system. |
| **Characterization of Dose Formulations:** | Assays to verify the stability, homogeneity, and concentration of each test substance in the vehicle will be the responsibility of the Sponsor and will not be contained in the final report. |
| **Disposition:** | Unused bulk test substance will be returned to the Sponsor. Unused dose formulations, not reserved for dose concentration analysis, will be discarded immediately after use in the test system. |
| **Test Substance Handling:** | The test substances and dose formulations will be handled with the use of eye protection, gloves, and a protective smock or laboratory coat. |

**Positive Controls without Activation**

| | |
|---|---|
| **For Strains TA1535 & TA100:** | Sodium azide |
| **CAS No.:** | 26628-22-8 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |
| **Physical Description:** | To be specified in the final report |
| **Storage Conditions:** | Room temperature, 15° to 30°C |
| **Dose/Plate:** | 5 µg/50 µl |

| | |
|---|---|
| **For Strain TA1537:** | 9-Aminoacridine hydrochloride |
| **CAS No.:** | 52417-22-8 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |
| **Physical Description:** | To be specified in the final report |
| **Storage Conditions:** | Room temperature, 15° to 30°C |
| **Dose/Plate:** | 50 µg/50 µl |
| | |
| **For Strain TA98:** | 2-Nitrofluorene |
| **CAS No.:** | 607-57-8 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |
| **Physical Description:** | To be specified in the final report |
| **Storage Conditions:** | Room temperature, 15° to 30°C |
| **Dose/Plate:** | 5 µg/50 µl |
| | |
| **For Strain WP2 (*uvr*A):** | 4-Nitroquinoline N-oxide |
| **CAS No.:** | 56-57-5 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |
| **Physical Description:** | To be specified in the final report |
| **Storage Conditions:** | Frozen, −20° to −10°C |
| **Dose/Plate:** | 2.5 µg/50 µl |
| | |
| **Positive Control with Activation** | |
| **Name:** | 2-Anthramine (2-Aminoanthracene) |
| **CAS No.:** | 613-13-8 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |
| **Physical Description:** | To be specified in the final report |
| **Storage Conditions:** | Room temperature, 15° to 30°C |
| **Dose/Plate:** | 2 µg/50 µl (TA98, TA100), 4 µg/50 µl (TA1535, TA1537) & 20 µg/50 µl [WP2 (*uvr*A)] in the presence of activation |
| **Characterization of Positive Controls:** | Characterization of each positive control substance will be obtained from the manufacturer's Certificate of Analysis. |
| | |
| **Solvent for the Positive Controls** | |
| **Name:** | Dimethyl sulfoxide (DMSO) |
| **CAS No.:** | 67-68-5 |
| **Manufacturer:** | To be specified in the final report |
| **Lot No.:** | To be specified in the final report |

| | |
|---|---|
| **Physical Description:** | Clear, colorless liquid |
| **Storage Conditions:** | Room temperature, 15° to 30°C |
| **Characterization of Solvent:** | Characterization of the solvent will be obtained from the manufacturer's Certificate of Analysis. |

## C. Test System

**Test System Justification.** Microbial mutagenicity assays are capable of rapidly detecting the mutagenic activity of many materials, including a wide range of chemical classes. Many chemicals that elicit a mutagenic response in the *Salmonella* assay have been shown to be potentially mutagenic and carcinogenic to humans and laboratory animals. One advantage of using the procedure with *E. coli* is that this strain has an A-T base-pair at the critical mutation site and thus is sensitive to some agents that are not detected by the *Salmonella* strains. Because microbial mutagenicity assays are short-term, sensitive, and reliable tests for assessing mutagenic potential, their use for genotoxic evaluation of chemicals is appropriate.

**Indicator Organisms**

| | |
|---|---|
| **Species:** | *Salmonella typhimurium* LT2 |
| **Strains:** | TA1535, TA1537, TA98, and TA100 |
| **Source:** | Dr. Bruce Ames, University of California, Berkeley |
| **Species:** | *Escherichia coli* |
| **Strain:** | WP2 (*uvr*A) |
| **Source:** | National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland |

**Description of the Strains.** The *Salmonella* tester strains have mutations in the histidine operon, a mutation that leads to a defective lipopolysaccharide coat (*rfa*), and a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (UV)-induced DNA damage (*uvr*B). The *rfa* mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The *uvr*B mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to histidine independence by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the drug resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most

mutagens (McCann et al., 1975). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker for detecting the presence of the plasmid in the cell (Mortelmans and Stocker, 1979). The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens such as ICR-191. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by adding the plasmid pKM101, which makes it more sensitive to some mutagenic agents (Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

The *E. coli* WP2 (*uvr*A) strain carries a mutation at the tryptophan (*trp*) allele, which is an auxotrophic mutation reverted by base-pair substitution. The strain is deficient in the repair of UV-induced DNA damage (*uvr*A) (Bridges, 1972; Green and Muriel, 1976; Mortelmans and Riccio, 2000) and thus has enhanced sensitivity to some mutagenic agents.

**Test System Identification:** The strains are analyzed for their genetic markers and for the presence of the plasmid whenever experiments are performed.

**Culture Conditions:** The indicator strains are kept frozen at -80°C in nutrient broth supplemented with 10% sterile glycerol. New frozen stock cultures are made from single colony isolates. Cultures are inoculated into 50 ml Oxoid Nutrient Broth No. 2 (CM 67) and allowed to sit unshaken for 2 to 4 hr, then gently shaken (100 to 125 rpm) for about 11 to 14 hr at 37°C.

**Identification:** Plates are labeled with indelible ink to identify the test substance, the strain, the dose level, and the presence or absence of the metabolic activation system.

**Metabolic Activation**

**Supplier:** Molecular Toxicology, Inc., Boone, NC

**Description:** Aroclor 1254-induced rat liver homogenate preparation (S9)

**Preparation:** Liver enzymes are induced by injecting adult male Sprague-Dawley rats with Aroclor 1254 (500 mg/kg) 5 days before they are sacrificed. The S9 consists of 9000 × *g* supernatant of liver homogenized in KCl (1 g wet weight of liver to

3 ml of 0.154M KCl).

**Quality Control:** Dilutions from each lot of S9, ranging from 0.2 to 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene and 2-aminoanthracene to intermediates mutagenic to TA100 prior to product release.

**Metabolic Activation Mixture:** The metabolic activation mixture (Ames et al., 1975; Maron and Ames, 1983) for the experiment(s) will consist of the components and amounts shown below.

**PREPARATION OF METABOLIC ACTIVATION MIXTURE FOR 50 ml**

| Ingredient | 5% S9 Mix (ml) | 10% S9 Mix (ml) |
|---|---|---|
| Rat liver S9 (Aroclor 1254-induced) | 2.5 | 5.0 |
| $MgCl_2$ (0.4 M) and KCl (1.65 M) salts | 1.0 | 1.0 |
| Glucose-6-phosphate (1 M) | 0.25 | 0.25 |
| NADP (0.1 M) | 2.0 | 2.0 |
| Sodium phosphate buffer (0.2 M, pH 7.4) | 25.0 | 25.0 |
| Sterile distilled water | 19.25 | 16.75 |

**D. Experimental Procedure**

To a sterile 13 × 100-mm test tube placed in a 43°C heating block will be added:

(1) 2 ml of molten top agar

(2) 0.1 ml of indicator organisms (about $10^8$ bacteria)

(3) appropriate amount of the test substance

(4) 0.5 ml of metabolic activation mixture or buffer.

This mixture will be stirred gently, and then poured onto plates containing about

25 ml of minimal glucose agar. After the top agar has set, the plates will be incubated at ~37°C for about 48 hr. The revertant colonies will be counted after the incubation period; however, if the plates cannot be immediately evaluated, they will be refrigerated at ~4°C until they can be counted.

Concurrent sterility, solvent, and positive controls will be performed with each experiment. Sterility controls will include separately plating out each test substance, metabolic activation mixture, and buffer. Solvent controls will be performed for the positive controls and will consist of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl DMSO, the solvent used to dissolve the positive control substances. The solvent control for the test substance, referred to as the zero dose, will consist of top agar, bacteria, metabolic activation mixture or buffer, and the solvent/diluent for the test substance. Positive controls will be performed with each strain and consist of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl of the positive control substance.

## E. Data Collection

**Control of Bias.** Bias is controlled by collecting data with an automated colony counter when possible.

**Colony Counting.** The revertant colonies will be counted using an automated colony counter, Sorcerer Image Analysis System (version 2.2) and the data managed through the Ames Study Manager (version 1.21), both manufactured by Perceptive Instruments (Suffolk, England). When accurate counts cannot be obtained (e.g., because of precipitation on the plates), the colonies will be counted manually using an electric probe colony counter.

## F. Evaluation of Data

**Criteria for Valid Assay.** An experiment is considered valid when solvent controls are within ± 10% of historical limits for spontaneous revertants, when positive control mutagens elicit a positive response (≥ 5-fold increase over the mean value for the solvent for the respective strain), and when there are at least three nontoxic dose levels (mutagenicity experiments). When experimental plates and sterility control plates indicate gross contamination, the results are not considered valid and the experiment is repeated. In addition, whenever experiments are performed, the strains are analyzed to confirm their genetic markers and the presence of the plasmid. If anomalies exist, the experiment is repeated.

**Statistical Methods.** (1) Means and standard deviation will be calculated from the individual plate counts; (2) Levene's test (Levene, 1960) will be performed to

determine if a significant difference exists among treatment variances; (3) treatments will be compared with controls by using a one-tailed Dunnett's *t*-test (Dunnett, 1980) and within-levels pooled variance; and (4) evaluation of dose-relatedness for all treatments will be made by regression analysis (Draper and Smith, 1981) of revertant counts versus the log of the concentrations (to allow inclusion of the zero dose, 1 will be added to the dose before calculating the log). The significance of the regression will be tested using a *t*-statistic.

**Criteria for Interpretation.** The following criteria will be used as guidelines for the interpretation of the data; however, the conclusions of the study will be based upon the Study Director's evaluation and interpretation of the data.

**Positive.** A test substance will be considered a mutagen when a reproducible and statistically significant ($p < 0.01$) increase in revertants is observed at one or more dose levels. A statistically significant ($p < 0.01$) dose-related increase in the number of revertants will also be considered a positive response.

**Negative.** A test substance will be considered a nonmutagen when the values for the dose levels are not reproducible or significant or when there is no statistically significant dose-related increase in the number of revertants.

**Inconclusive.** When a test substance cannot be identified clearly as a mutagen or nonmutagen, the results will be classified as inconclusive.

## VII. REGULATORY COMPLIANCE

### A. Good Laboratory Practice (GLP) Compliance

This study will be conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP), with the exceptions that the characterization of the test substances (identity, purity, and stability) and the supporting analytical chemistry of the dose formulations will not be provided to the testing facility as this testing will not be performed by the Sponsor nor the testing facility.

### B. Standard Operating Procedures

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the Standard Operating Procedures of the laboratory, and any deviations will be documented.

### C. Protocol Amendments

All changes in or revisions of an approved protocol and the reasons for them will be documented and signed and dated by the Study Director and the Sponsor's Representative. Amendments will be maintained with the protocol. Verbal approval for changes in the protocol may be granted by the Sponsor's Representative, but a written amendment will follow.

### D. Retention of Records and Study Samples

All raw data, the original protocol and final report, relevant documents, and records specific to this study are the property of the Sponsor and will be stored at SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025. All records will be maintained for at least 10 years. At the end of the retention period, the Sponsor will be contacted regarding further disposition of these records. Wet specimens (e.g., colonies in agar) and samples of the control substances are not required to be retained.

## VIII. REPORTING

The final report will describe the study design, procedures, and findings and will present an analysis and summary of the data followed by the conclusions derived from the analyses. A draft report will be issued prior to submission of the final report.

## IX. BIBLIOGRAPHY

Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat. Res. **31**: 347-364.

Bridges, B. A. 1972. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. **21**: 413-416.

Draper, N. R., and H. Smith, Jr. 1981. *Applied Regression Analysis*, 2nd ed. John Wiley & Sons, Inc., New York.

Dunnett, C. W. 1980. Pairwise multiple comparisons in the homogeneous variance unequal sample size case. J. Am. Stat. Assoc. **75**: 372.

Green, M.H.L., and W. J. Muriel. 1976. Mutagen testing using $trp^+$ reversion in *Escherichia coli*. Mutat. Res. **38**: 3-32.

Levene, H. 1960. Robust tests for equality of variance. In *Contributions to Probability*

*and Statistics*, I. Olkin, Ed. Stanford University Press, Stanford, CA, pp. 278-292.

Maron, D. M., and B. N. Ames. 1983. Revised methods for the *Salmonella* mutagenicity test. Mutat. Res. **113**: 173-215.

McCann, J., N. E. Spingarn, J. Kobori, and B. N. Ames. 1975. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Natl. Acad. Sci. USA **72**: 979-983.

Mortelmans, K. E., and B.A.D. Stocker. 1979. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genet. **167**: 317-327.

Mortelmans, K., E.S. Riccio. The bacterial tryptophan reverse mutation assay with *Escherichic coli* WP2. Mutat. Res. **455**: 61-69, 2000.

Mortelmans, K., E. Zeiger. The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res. **455**: 29-60, 2000.

# Protocol Amendment No. 1

**PROTOCOL TITLE:** **Evaluation of Two HRJ Fuels in the *Salmonella-Escherichia coli*/Microsome Plate Incorporation Assay**

**SRI Study Number:** G371-11

**Sponsor:** Henry M. Jackson Foundation for the Advancement of Military Medicine

**Sponsor's Representative:** David R. Mattie, PhD, DABT

**SRI Study Director:** Edward S. Riccio, BS

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**VI. MATERIALS AND METHODS, A. Experimental Design, Page 2 of 12:**

Add to the protocol, the following dose levels to be used for the first experiment for mutagenicity.

***Based on information derived from the range-finding experiment, the first experiment for mutagenicity with HRJ Camelina and HRJ Tallow will be conducted at doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate in the presence and absence of metabolic activation containing 5% S9.***

**Reason for Change:** This addition to the protocol is necessary to establish the dose levels for the first mutagenicity experiment based on the results of the range-finding experiment.

**Effect on the Study:** These dose levels should allow for the assessment of potential mutagenicity and contain at least three nontoxic dose levels.

**APPROVALS**

David R. Mattie — 14 Feb 11

David R. Mattie, PhD, DABT
Sponsor's Authorized Representative — Date

Edward S. Riccio — 2/14/2011

Edward S. Riccio
SRI Study Director — Date

# Protocol Amendment No. 2

| | |
|---|---|
| **PROTOCOL TITLE:** | **Evaluation of Two HRJ Fuels in the *Salmonella-Escherichia coli*/Microsome Plate Incorporation Assay** |
| **SRI Study Number:** | G371-11 |
| **Sponsor:** | Henry M. Jackson Foundation for the Advancement of Military Medicine |
| **Sponsor's Representative:** | David R. Mattie, PhD, DABT |
| **SRI Study Director:** | Edward S. Riccio, BS |

This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**VI. MATERIALS AND METHODS, A. Experimental Design, Page 2 of 12:**

Add to the protocol, the following dose levels to be used for the second experiment for mutagenicity.

***Based on information derived from the first experiment for mutagenicity, the second experiment for mutagenicity with HRJ Camelina and HRJ Tallow will be conducted at doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate in the presence and absence of metabolic activation containing 10% S9.***

**Reason for Change:** This addition to the protocol is necessary to establish the test conditions to be used in the second mutagenicity experiment.

**Effect on the Study:** These dose levels should allow for the assessment of potential mutagenicity and contain at least three nontoxic dose levels.

**APPROVALS**

David R. Mattie — 11 Mar 11

David R. Mattie, PhD, DABT
Sponsor's Authorized Representative — Date

Edward S. Riccio — 3/11/2011

Edward S. Riccio
SRI Study Director — Date

# APPENDIX C. DERMAL IRRITATION STUDY ANIMAL ROOM ENVIRONMENTAL CONDITIONS

ACUTE DERMAL STUDY OF BIO-BASED JET FUELS IN RABBITS

PROJECT NO.:WIL- 773002 TEMPERATURE/HUMIDITY - STUDY SUMMARY REPORT

SPONSOR: 773 - H M JACKSON FOUNDATION



STUDY SPECIFICATIONS: 773002 DATE IN 01/18/11 TIME IN 08:00

DATE OUT 02/01/11 TIME OUT 16:00

ROOM SPECIFICATIONS: B ROOM 106 LOW TEMPERATURE °F: 61.0 HIGH TEMPERATURE °F: 71.0 LOW HUMIDITY %RH: 30.0

TEST SYSTEM: RABBIT LOW TEMPERATURE °C: 16.1 HIGH TEMPERATURE °C: 21.7 HIGH HUMIDITY %RH: 70.0

| | PRIMARY TEMP | | SECONDARY TEMP | | PRIMARY HUM | SECONDARY HUM |
|---|---|---|---|---|---|---|
| DATE | MEAN (°F) | MEAN (°C) | MEAN (°F) | MEAN (°C) | MEAN (%RH) | MEAN (%RH) |
| 01/18/11 | 65.9 | 18.8 | 65.7 | 18.7 | 53.8 | 54.0 |
| 01/19/11 | 65.7 | 18.7 | 65.4 | 18.6 | 45.4 | 45.7 |
| 01/20/11 | 65.3 | 18.5 | 64.8 | 18.2 | 42.8 | 44.3 |
| 01/21/11 | 65.5 | 18.6 | 65.1 | 18.4 | 33.5 | 34.2 |
| 01/22/11 | 65.6 | 18.7 | 65.2 | 18.4 | 30.6 | 31.1 |
| 01/23/11 | 65.4 | 18.6 | 65.1 | 18.4 | 31.0 | 31.4 |
| 01/24/11 | 65.5 | 18.6 | 65.1 | 18.4 | 39.3 | 40.1 |
| 01/25/11 | 65.5 | 18.6 | 65.3 | 18.5 | 49.4 | 50.3 |
| 01/26/11 | 65.4 | 18.6 | 65.2 | 18.4 | 47.0 | 47.7 |
| 01/27/11 | 65.6 | 18.7 | 65.4 | 18.6 | 46.5 | 46.9 |
| 01/28/11 | 65.6 | 18.7 | 65.4 | 18.6 | 46.7 | 47.4 |
| 01/29/11 | 65.6 | 18.7 | 65.2 | 18.4 | 46.4 | 47.1 |
| 01/30/11 | 65.6 | 18.7 | 65.3 | 18.5 | 47.0 | 47.5 |
| 01/31/11 | 65.8 | 18.8 | 65.4 | 18.6 | 38.5 | 38.7 |
| 02/01/11 | 65.2 | 18.4 | 64.8 | 18.2 | 45.4 | 45.6 |

| | PRIMARY TEMP | | SECONDARY TEMP | | PRIMARY HUM | SECONDARY HUM |
|---|---|---|---|---|---|---|
| DATE | MEAN (°F) | MEAN (°C) | MEAN (°F) | MEAN (°C) | MEAN (%RH) | MEAN (%RH) |

| **SUMMARY OF DAILY MEANS** | MEAN | MIN | MAX |
|---|---|---|---|
| PRIMARY TEMP °F: | 65.6 | 65.2 | 65.9 |
| PRIMARY TEMP °C: | 18.7 | 18.4 | 18.8 |
| SECONDARY TEMP °F: | 65.2 | 64.8 | 65.7 |
| SECONDARY TEMP °C: | 18.4 | 18.2 | 18.7 |
| PRIMARY HUM %RH: | 42.6 | 30.6 | 53.8 |
| SECONDARY HUM %RH: | 43.1 | 31.1 | 54.0 |
| N DAYS | 15 | | |

**B ROOM 106 SUMMARY OF HOURLY VALUES**

| | PRIMARY TEMP | | | | SECONDARY TEMP | | | | PRIMARY HUM | | SECONDARY HUM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MEAN | 65.6 | °F | 18.7 | °C | 65.2 | °F | 18.4 | °C | 42.6 | %RH | 43.1 | %RH |
| MIN | 62.4 | °F | 16.9 | °C | 62.5 | °F | 16.9 | °C | 26.9 | %RH | 27.3 | %RH |
| MAX | 67.5 | °F | 19.7 | °C | 66.9 | °F | 19.4 | °C | 58.2 | %RH | 64.9 | %RH |
| SD | 0.63 | | 0.35 | | 0.64 | | 0.36 | | 7.36 | | 7.50 | |
| SE | 0.03 | | 0.02 | | 0.03 | | 0.02 | | 0.40 | | 0.41 | |
| N SAMPLES | 342 | | | | 342 | | | | 341 | | 341 | |
| FIRST DAY | 01/18/11 | | | | | | | | | | | |
| LAST DAY | 02/01/11 | | | | | | | | | | | |
| N DAYS | 15 | | | | | | | | | | | |

**STUDY 773002 SUMMARY OF HOURLY VALUES**

| | PRIMARY TEMP | | SECONDARY TEMP | | PRIMARY HUM | SECONDARY HUM |
|---|---|---|---|---|---|---|
| MEAN | 65.6 °F | 18.7 °C | 65.2 °F | 18.4 °C | 42.6 %RH | 43.1 %RH |
| MIN | 62.4 °F | 16.9 °C | 62.5 °F | 16.9 °C | 26.9 %RH | 27.3 %RH |
| MAX | 67.5 °F | 19.7 °C | 66.9 °F | 19.4 °C | 58.2 %RH | 64.9 %RH |
| SD | 0.63 | 0.35 | 0.64 | 0.36 | 7.36 | 7.50 |
| SE | 0.03 | 0.02 | 0.03 | 0.02 | 0.40 | 0.41 |
| N SAMPLES | 342 | | 342 | | 341 | 341 |
| FIRST DAY | 01/18/11 | | | | | |
| LAST DAY | 02/01/11 | | | | | |
| N DAYS | 15 | | | | | |

# APPENDIX D. INDIVIDUAL DERMAL IRRITATION SCORES

**INDIVIDUAL DERMAL SCORES** **PAGE 1**

Material: JP-8, Occluded
Site: 0.5 mL/Site (4-Hour Exposure)

| | | | Erythema | | | | | | | Edema | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 60224-08 | M | A | 0 | 1 | 1 | 2 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 61517 | M | B | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60223-09 | M | C | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 3 + 3 + 4) / 12 + (0 + 0 + 0 + 0) / 12

PII = 10 / 12 + 0 / 12

PII = 0.8 + 0

PII = 0.8 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations: Head
A C
B D
Tail

Material: JP-8, Semi-occluded
Site: 0.5 mL/Site (4-Hour Exposure)

| | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 61523 | M | D | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60201-01 | M | A | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60222-06 | M | B | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (2 + 2 + 2 + 3) / 12 + (0 + 0 + 0 + 0) / 12

PII = 9 / 12 + 0 / 12

PII = 0.8 + 0

PII = 0.8 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:

Head
A C

B D
Tail

Material: HRJ Camelina, Occluded

Site: 0.5 mL/Site (4-Hour Exposure)

| | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 60224-08 | M | B | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 61517 | M | C | 0 | 1 | 1 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60223-09 | M | D | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 3 + 3 + 4) / 12 + (0 + 0 + 0 + 0) / 12

PII = 11 / 12 + 0 / 12

PII = 0.9 + 0

PII = 0.9 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:

Head

A C

B D

Tail

Material: HRJ Camelina, Semi-occluded
Site: 0.5 mL/Site (4-Hour Exposure)

| Group 2 | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 61523 | M | A | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60201-01 | M | B | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60222-06 | M | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 2 + 2 + 2) / 12 + (0 + 0 + 0 + 0) / 12

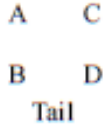
PII = 7 / 12 + 0 / 12

PII = 0.6 + 0

PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:



Material: HRJ Tallow, Occluded
Site: 0.5 mL/Site (4-Hour Exposure)

| Group 1 | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 60224-08 | M | C | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 61517 | M | D | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60223-09 | M | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 3 + 2 + 2) / 12 + (0 + 0 + 0 + 0) / 12

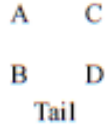
PII = 7 / 12 + 0 / 12

PII = 0.6 + 0

PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:



Material: HRJ Tallow, Semi-occluded
Site: 0.5 mL/Site (4-Hour Exposure)

| Group 2 | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 61523 | M | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60201-01 | M | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60222-06 | M | D | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 1 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 2 / 12 + 0 / 12

PII = 0.2 + 0

PII = 0.2 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:

Head
A C
B D
Tail

Material: HRJ Animal Fats and Oils, Occluded
Site: 0.5 mL/Site (4-Hour Exposure)

| Group 1 | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 60224-08 | M | D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 61517 | M | A | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60223-09 | M | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 1 + 1 + 1) / 12 + (0 + 0 + 0 + 0) / 12

PII = 3 / 12 + 0 / 12

PII = 0.3 + 0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:

Head
A C
B D
Tail

Material: HRJ Animal Fats and Oils, Semi-occluded

Site: 0.5 mL/Site (4-Hour Exposure)

| Group 2 | | | Erythema | | | | | | | Edema | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Sex | Site | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D | 0.5-1H | 24H | 48H | 72H | 4D | 7D | 14D |
| 61523 | M | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60201-01 | M | D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60222-06 | M | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 0 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 0 / 12 + 0 / 12

PII = 0 + 0

PII = 0 = Nonirritating

M = Male; H = Hours; D = Day

Site Locations:

Head

A C

B D

Tail

## APPENDIX E. SIGNATURE PAGES AND QUALITY ASSURANCE STATEMENTS

### ACUTE DERMAL IRRITATION STUDY OF HRJ CAMELINA, HRJ TALLOW AND HRJ ANIMAL FATS AND OILS BIO-BASED JET FUELS IN NEW ZEALAND WHITE RABBITS

**Compliance Statement**

This study, designated WIL-773002, was conducted in compliance with the U.S. EPA GLP Standards (40 CFR Part 792), 18 September 1989; the WIL Research SOPS; and the protocol as approved by the Sponsor. Analytical confirmation of the concentration, purity, homogeneity, and stability of the test substances was not supplied by the Sponsor and was not conducted as part of this study.

Jonathan M. Hurley, BS
Project Specialist, General Toxicology
Study Director

01 Apr 2011
Date

**Report Review and Approval**

Approved and Submitted By:

Jonathan M. Hurley, BS
Project Specialist, General Toxicology
Study Director

01 Apr 2011
Date

Report Prepared By:

Chad P. Durastanti, BS
Associate Study Analyst

1 April 2011
Date

Reviewed By:

Charlene A. Weygandt, BS
Lead Analyst and Scientific Advisor,
Reporting & Technical Support Services

1 April 2011
Date

for Thomas P. O'Neill, BS, DABT
Assistant Director, General Toxicology

1 Apr. 2011
Date

**Quality Assurance Unit Statement**

*Phases Inspected*

| Date(s) of Inspection(s) | Phase Inspected | Date(s) Findings Reported to Study Director | Date(s) Findings Reported to Management | Auditor(s) |
|---|---|---|---|---|
| 18-Jan-2011<br>19-Jan-2011 | Test Article Administration | 19-Jan-2011 | 28-Feb-2011 | R.Rohr |
| 08-Feb-2011 | Study Records (Rx-1) | 09-Feb-2011 | 23-Mar-2011 | R.Siburt |
| 09-Feb-2011<br>14-Feb-2011<br>16-Feb-2011 | Study Records (I-1) | 16-Feb-2011 | 23-Mar-2011 | R.Siburt |
| 08-Mar-2011<br>09-Mar-2011<br>10-Mar-2011 | Draft Report | 10-Mar-2011 | 01-Apr-2011 | R.Siburt |

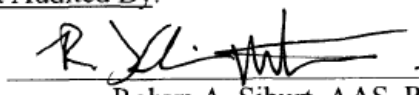
This study was inspected in accordance with the US EPA GLP Standards (40 CFR Part 792), the WIL Research SOPs, and the Sponsor's protocol and protocol amendments. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. Review of the protocol and protocol amendments (if applicable) as well as a yearly internal facility inspection are conducted by the WIL Research Quality Assurance Unit. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and the WIL Research SOPs.

*Approval*

This study was inspected according to the criteria discussed above.

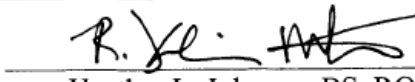
Report Audited By:



Robyn A. Siburt, AAS, RLAT
Compliance Specialist

1 Apr 2011
Date

Report Released By:



Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

1 Apr 2011
Date

**EVALUATION OF TWO HRJ FUELS IN THE *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY**

**Report Review and Approval**

Written by:

Edward S. Riccio, BS
Study Director

6/17/2011
Date

Approved by:

Carol E. Green, PhD, DABT
Senior Director, Toxicology and Pharmacokinetics

6-17-11
Date

**Good Laboratory Practice Compliance Statement**

The study with Two HRJ Fuels, Camelina (UOP) and HRJ Tallow (UOP), submitted by the Sponsor, Henry Jackson Foundation for the Advancement of Military Medicine, was conducted in compliance with the United States Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR Part 792, with the following exceptions:

Characterization of the test substances (identity, purity, and stability) and the supporting analytical chemistry of the dose formulations were not provided to the testing facility as this testing was not performed by the Sponsor or the testing facility.

Edward S. Riccio, BS
Study Director

6/17/2011
Date

David R. Mattie, PhD, DABT
Sponsor's Representative

15 Jun 11
Date

**Quality Assurance Unit: Final Report and Conflict of Interest Statement**

SRI's Quality Assurance Unit assures that the study-- Evaluation of Two HRJ Fuels in the *Salmonella -Escherichia coli*/Microsome Plate Incorporation Assay, SR I Study Number G371-11, has been reviewed for adherence to U. S. Environmental Protection Agency (EPA) under Good Laboratory Practice Standards (40 CFR Part 792).

The following inspections were conducted during this study:

| Phase Inspected | Date of Inspection | Date Findings Reported to Management/Study Director |
|---|---|---|
| Protocol | 01-20-11 | 01-20-11 |
| Counting | 02-17-11 | 02-17-11 |
| Dose Preparation | 03-01-11 | 03-01-11 |
| Plating | 03-16-11 | 03-16-11 |
| Raw Data | 04-27-11 | 04-27-11 |
| Draft Final Report | 04-27-11 | 04-27-11 |
| Final Report Verification | 06-17-11 | 06-17-11 |

This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Beverly Beatty Noah Fishlock Jeannette Robinson Thomas Bregante

Beverly Beatty, RQAP-GLP
SRI Quality Assurance Unit

06/17/11
Date

**Key Personnel**

| Name | Functional Role |
|---|---|
| Carol E. Green, PhD, DABT | Senior Director, Toxicology and Pharmacokinetics |
| Mike Hwang, BS | Research Associate |
| Lisa M. Jack, MA | Statistician |
| Linh Nguyen, BS | Supervisor, Dose Preparation |
| Edward S. Riccio, BS | Study Director |

# APPENDIX F. CERTIFICATES OF ANALYSIS

G371-11

Mallinckrodt CHEMICALS

# Dimethyl Sulfoxide

## AR® (ACS)

**Product No. 4948**
**Lot No. J12J03**
**Release Date 03/16/2010**

## Certificate of Analysis

| TEST | SPECIFICATION | RESULT |
|---|---|---|
| Meets A.C.S. Specifications | | |
| Assay ($(CH_3)_2SO$) (by GC, corrected for water) | 99.9 % min. | 99.9 % |
| Appearance (clear, colorless liquid) | Passes Test | Passes Test |
| Residue after Evaporation | 0.01 % max. | < 0.001 % |
| Titrable Acid (meq/g) | 0.001 max. | < 0.0002 |
| Water ($H_2O$)(by coulometry) | 0.1 % max. | 0.04 % |

For Laboratory, Research or Manufacturing Use

Country of Origin: USA

ISO

For questions on this Certificate of Analysis please contact Technical Services at 1-800-582-2537 or 908-859-2151
Mallinckrodt Baker, Inc. • 222 Red School Lane • Phillipsburg, NJ 08865 • Phone: 908.859.2151 • Fax: 908.859.6905



True copy of original.

Original located: Chemical Receipt Binder
Initials: VC Date: 3/22/11

G371-11

# Certificate of Analysis

SIGMA-ALDRICH

| | |
|---|---|
| **Product Name** | 2-Nitrofluorene, 98% |
| **Product Number** | N16754 |
| **Product Brand** | ALDRICH |
| **CAS Number** | 607-57-8 |
| **Molecular Formula** | $C_{13}H_9NO_2$ |
| **Molecular Weight** | 211.22 |

| **TEST** | **LOT s43858 RESULTS** |
|---|---|
| **QC Acceptance date** | 14-AUG-2007 |
| **APPEARANCE - COLOUR** | YELLOW-TAN |
| **APPEARANCE - STATE** | POWDER |
| **ELEMENTAL ANALYSIS - CARBON** | 74.0% |
| **ELEMENTAL ANALYSIS - HYDROGEN** | 4.3% |
| **ELEMENTAL ANALYSIS - NITROGEN** | 6.6% |
| **HPLC - PURITY** | 97.9% |
| **IR SPECTROSCOPY - FTIR SPECTRUM** | CONFORMS TO STRUCTURE |

Claudia Mayer

Claudia Mayer, Manager
Quality Control
Steinheim Germany

G371-11

# Certificate of Analysis

SIGMA-ALDRICH

| | |
|---|---|
| **Product Name** | 4-Nitroquinoline *N*-oxide |
| **Product Number** | N8141 |
| **Product Brand** | ALDRICH |
| **CAS Number** | 56-57-5 |
| **Molecular Formula** | $C_9H_6N_2O_3$ |
| **Molecular Weight** | 190.16 |

| TEST | SPECIFICATION | LOT 090m1161v RESULTS |
|---|---|---|
| **Appearance (Color)** | Yellow to Brown | Yellow |
| **Appearance (Form)** | Powder | Powder |
| **Solubility (Turbidity)** | Clear to Slightly Hazy<br>25 mg/mL, Acetone | Clear |
| **Solubility (Color)** | Yellow to Orange | Yellow-Orange |
| **Carbon** | 55.4 - 58.3 % | 56.9 % |
| **Nitrogen** | 14.4 - 15.1 % | 14.8 % |
| **Purity (HPLC)** | ≥98 % | 99 % |
| **Specification Date:** | | SEP 2010 |
| **Date of QC Release:** | | SEP 2010 |
| **Print Date:** | | SEP 23 2010 |

Rodney Burbach

Rodney Burbach, Manager
Quality Control
St. Louis, Missouri USA

G371-11

# Certificate of Analysis

SIGMA-ALDRICH

| | |
|---|---|
| **Product Name** | 9-Aminoacridine hydrochloride monohydrate, 98% |
| **Product Number** | A38401 |
| **Product Brand** | ALDRICH |
| **CAS Number** | 52417-22-8 |
| **Molecular Formula** | $C_{13}H_{10}N_2 \cdot HCl \cdot H_2O$ |
| **Molecular Weight** | 248.71 |

| TEST | SPECIFICATION | LOT 07620td RESULTS |
|---|---|---|
| APPEARANCE | YELLOW OR YELLOW-GREEN POWDER | YELLOW POWDER |
| INFRARED SPECTRUM | CONFORMS TO STRUCTURE. | CONFORMS TO STRUCTURE. |
| TITRATION | 97.5% - 102.5% (WITH AGNO3) | 98.7% (WITH SILVER NITRATE) |
| HIGH PRESSURE LIQUID CHROMATOGRAPHY | 97.5% (MINIMUM) | 99.9% |
| TITRATION | TYPICALLY 3%-8% H2O (WITH "KARL FISCHER RGT) | 7.0% H2O (WITH "KARL FISCHER" REAGENT) |
| PRODUCT CROSS REFERENCE INFORMATION | | REPLACES PRODUCT NUMBER A1135 |
| QUALITY CONTROL ACCEPTANCE DATE | | DECEMBER 2005 |

Barbara Rajzer

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

G371-11

# Certificate of Analysis

SIGMA-ALDRICH

| | |
|---|---|
| **Product Name** | Sodium azide, *ReagentPlus*®, ≥99.5% |
| **Product Number** | S2002 |
| **Product Brand** | SIAL |
| **CAS Number** | 26628-22-8 |
| **Molecular Formula** | $NaN_3$ |
| **Molecular Weight** | 65.01 |

| TEST | SPECIFICATION | LOT mkbf6507v RESULTS |
|---|---|---|
| Appearance (Color) | White | White |
| Appearance (Form) | Powder | Powder |
| Purity (Titration by NaOH) | ≥99.5 % | 99.5 % |
| Recommended Retest Period | ---------- | ---------- |
| | 5 Years | |
| Specification Date: | | OCT 2010 |
| Date of QC Release: | | OCT 2010 |
| Recommended Retest Date: | | OCT 2015 |
| Print Date: | | NOV 08 2010 |

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

3371-11

SIGMA-ALDRICH

ALDRICH

Riedstrasse 2, D-89555 Steinheim/Germany
Tel: +49 73 2997 2550 Fax: +49 73 2997 2557

# Certificate of Analysis

| | |
|---|---|
| **Product Name:** | 2-AMINOANTHRACENE<br>96 % |
| **Product Number:** | A38800 |
| **Product Brand:** | Aldrich |
| **Molecular Formula:** | $C_{14}H_{11}N$ |
| **Molecular Mass:** | 193.24 |
| **CAS Number:** | 613-13-8 |

| TEST | SPECIFICATION | LOT STBB1901 RESULTS |
|---|---|---|
| APPEARANCE (COLOR) | GOLD TO TAN TO OLIVE-GREEN | OLIVE GREEN |
| APPEARANCE (FORM) | POWDER | POWDER |
| PURITY (HPLC AREA %) | ≥ 95.5 % | 97.5 % |
| SOLUBILITY (COLOR) | YELLOW TO GREEN TO BROWN | DARK GREEN |
| SOLUBILITY (TURBIDITY) | CLEAR TO OPAQUE | OPAQUE |
| SOLUBILITY (METHOD) | 50MG/ML DMF | 50MG/ML DMF |
| CARBON CONTENT | 83.1 - 91.8 % | 86.8 % |
| NITROGEN CONTENT | 6.9 - 7.6 % | 7.2 % |
| INFRARED SPECTRUM | CONFORMS TO STRUCTURE | CONFORMS |

**QC RELEASE DATE** 09/DEC/09

Claudia Mayer

Claudia Mayer, Manager
Quality Control
Steinheim, Germany

# APPENDIX G. INDIVIDUAL AND MEAN COUNTS - MICROSOME PLATE INCORPORATION ASSAY

## Table 1
## INDIVIDUAL AND MEAN PLATE COUNTS
## RANGE-FINDING EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-11 Camelina (UOP)**
**Experiment: G371-11 Range Finder**
Assay Conditions: Plate incorporation assay

Study Code: G371-11
Date Plated: 2/8/2011
Date Counted: 2/10/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA100** | **HRJ Camelina** | 0.156 µl | *90.0* | *8.9* | *0.8* | 87 N, 100 N, 83 N |
| | | 0.313 µl | *100.3* | *5.7* | *0.9* | 105 N, 102 N, 94 N |
| | | 0.625 µl | *89.0* | *8.0* | *0.8* | 97 N, 81 N, 89 N |
| | | 1.25 µl | *86.3* | *8.0* | *0.8* | 94 N, 78 N, 87 N |
| | | 2.5 µl | *84.3* | *3.1* | *0.8* | 81 N, 87 N, 85 N |
| | | 5µl | *70.7* | *9.5* | *0.7* | 78 N, 60 N, 74 N |
| | **DMSO** | | *108.0* | *7.2* | | 102, 106, 116 |
| | **Untreated Control** | | *128.6* | *24.2* | | 127, 109, 102, 145, 160 |
| **TA100** | **SA** | 5 µg | *1966.0* | *17.7* | *18.2* | 1950, 1985, 1963 |
| **TA100** | **DMSO** | -- | *105.0* | *7.0* | *1.0* | 105, 98, 112 |

Key to Positive Controls

| | |
|---|---|
| SA | Sodium Azide |
| DMSO | Dimethyl Sulfoxide |

**With metabolic activation (5% S-9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA100** | **HRJ Camelina** | 0.156 µl | *120.0* | *9.5* | *1.0* | 111 N, 130 N, 119 N |
| | | 0.313 µl | *116.0* | *1.0* | *1.0* | 117 N, 115 N, 116 N |
| | | 0.625 µl | *94.3* | *3.5* | *0.8* | 94 N, 98 N, 91 N |
| | | 1.25 µl | *126.0* | *15.1* | *1.1* | 112 N, 142 N, 124 N |
| | | 2.5 µl | *122.3* | *9.0* | *1.1* | 128 N, 127 N, 112 N |
| | | 5µl | *173.3* | *11.9* | *1.5* | 165 N, 168 N, 187 N |
| | **DMSO** | | *115.3* | *12.5* | | 101, 121, 124 |
| **TA100** | **2AN (5% S9)** | 2 µg | *1606.3* | *68.9* | *13.9* | 1542, 1598, 1679 |
| **TA100** | **DMSO (+S9)** | -- | *124.7* | *19.5* | *1.1* | 111, 116, 147 |

Key to Positive Controls

| | |
|---|---|
| 2AN (5% S9) | 2-Aminoanthracene (5% S9) |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background lawn |

## Table 1 (concluded)
## INDIVIDUAL AND MEAN PLATE COUNTS
## RANGE-FINDING EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-11 Tallow** Study Code: G371-11
**Experiment: G371-11 Range Finder** Date Plated: 2/8/2011
Assay Conditions: Plate incorporation assay Date Counted: 2/10/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA100** | **HRJ Tallow** | 0.156 µl | *89.7* | *4.5* | *0.8* | 90 N, 85 N, 94 N |
| | | 0.313 µl | *111.0* | *8.5* | *1.0* | 112 N, 119 N, 102 N |
| | | 0.625 µl | *106.3* | *23.9* | *1.0* | 117 N, 123 N, 79 N |
| | | 1.25 µl | *75.0* | *10.4* | *0.7* | 87 N, 68 N, 70 N |
| | | 2.5 µl | *79.0* | *10.4* | *0.7* | 74 N, 72 N, 91 N |
| | | 5µl | *80.3* | *14.4* | *0.7* | 91 N, 64 N, 86 N |
| | **DMSO** | | *108.0* | *7.2* | | 102 N, 106 N, 116 N |
| | **Untreated Control** | | *128.6* | *24.2* | | 127, 109, 102, 145, 160 |
| **TA100** | **SA** | 5 µg | *1966.0* | *17.7* | *18.2* | 1950, 1985, 1963 |
| **TA100** | **DMSO** | -- | *105.0* | *7.0* | *1.0* | 105, 98, 112 |

Key to Positive Controls

| | |
|---|---|
| SA | Sodium Azide |
| DMSO | Dimethyl Sulfoxide |

**With metabolic activation (5% S-9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA100** | **HRJ Tallow** | 0.156 µl | *121.3* | *9.0* | *1.1* | 127 N, 126 N, 111 N |
| | | 0.313 µl | *132.7* | *7.8* | *1.2* | 139 N, 135 N, 124 N |
| | | 0.625 µl | *104.0* | *7.5* | *0.9* | 96 N, 105 N, 111 N |
| | | 1.25 µl | *115.3* | *17.4* | *1.0* | 109 N, 102 N, 135 N |
| | | 2.5 µl | *102.0* | *2.0* | *0.9* | 102 N, 104 N, 100 N |
| | | 5µl | *122.3* | *4.2* | *1.1* | 127 N, 119 N, 121 N |
| | **DMSO** | | *115.3* | *12.5* | | 101, 121, 124 |
| **TA100** | **2AN (5% S9)** | 2 µg | *1606.3* | *68.9* | *13.9* | 1542, 1598, 1679 |
| **TA100** | **DMSO (+S9)** | -- | *124.7* | *19.5* | *1.1* | 111, 116, 147 |

Key to Positive Controls

| | |
|---|---|
| 2AN (5% S9) | 2-Aminoanthracene (5% S9) |
| DMSO (+S9) | Dimethyl Sulfoxide +S9 |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background lawn |

**Table 2**
**STATISTICAL ANALYSIS OF THE FIRST MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 1 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Camelina (UOP) | 0 | 0 µl | 15 ± 3 | 11 ± 1 | 32 ± 2 | 142 ± 1 | 38 ± 2 |
| | 0 | 0.156 µl | 21 ± 3 | 13 ± 4 | 38 ± 3 | 136 ± 8 | 21 ± 2 |
| | 0 | 0.313 µl | 23 ± 4* | 14 ± 2 | 27 ± 2 | 145 ± 9 | 21 ± 6 |
| | 0 | 0.625 µl | 14 ± 2 | 12 ± 2 | 23 ± 6 | 112 ± 8 | 25 ± 2 |
| | 0 | 1.25 µl | 19 ± 4 | 12 ± 3 | 24 ± 4 | 124 ± 10 | 24 ± 5 |
| | 0 | 2.5 µl | 13 ± 2 | 11 ± 4 | 24 ± 5 | 145 ± 7 | 23 ± 1 |
| | 0 | 5 µl | 13 ± 3 | 11 ± 1 | 27 ± 4 | 118 ± 8 | 29 ± 5 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | -8.515 (3.508) | -2.405 (2.077) | -9.174 (4.721) | -17.691 (11.682) | -1.968 (5.613) |
| Y intercept (Standard error) | | | 19.323 (1.383) | 12.805 (0.819) | 30.613 (1.861) | 136.937 (4.605) | 26.486 (2.213) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 2 (continued)**
**STATISTICAL ANALYSIS OF THE FIRST MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 1 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Camelina (UOP) | 5 | 0 µl | 16 ± 3 | 13 ± 2 | 40 ± 5 | 157 ± 4 | 31 ± 2 |
| | 5 | 0.156 µl | 14 ± 2 | 11 ± 1 | 42 ± 5 | 133 ± 3 | 29 ± 4 |
| | 5 | 0.313 µl | 12 ± 0 | 12 ± 3 | 34 ± 5 | 127 ± 17 | 37 ± 4 |
| | 5 | 0.625 µl | 15 ± 4 | 12 ± 2 | 36 ± 6 | 139 ± 6 | 36 ± 5 |
| | 5 | 1.25 µl | 17 ± 1 | 11 ± 1 | 29 ± 2 | 147 ± 13 | 27 ± 4 |
| | 5 | 2.5 µl | 10 ± 2 | 13 ± 2 | 30 ± 4 | 150 ± 6 | 33 ± 5 |
| | 5 | 5 µl | 16 ± 3 | 10 ± 0 | 34 ± 5 | 143 ± 9 | 38 ± 7 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | -0.767 (2.592) | -2.273 (1.498) | -10.170 (4.652) | 6.658 (10.556) | 5.803 (4.700) |
| Y intercept (Standard error) | | | 14.465 (1.022) | 12.528 (0.591) | 38.098 (1.834) | 140.463 (4.162) | 31.287 (1.853) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 2 (continued)**
**STATISTICAL ANALYSIS OF THE FIRST MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 1 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Tallow (UOP) | 0 | 0 µl | 15 ± 3 | 11 ± 1 | 32 ± 2 | 142 ± 1 | 38 ± 2 |
| | 0 | 0.156 µl | 14 ± 2 | 12 ± 1 | 24 ± 5 | 130 ± 4 | 19 ± 1 |
| | 0 | 0.313 µl | 14 ± 1 | 15 ± 1 | 21 ± 5 | 134 ± 4 | 22 ± 3 |
| | 0 | 0.625 µl | 14 ± 2 | 10 ± 2 | 22 ± 1 | 129 ± 5 | 24 ± 1 |
| | 0 | 1.25 µl | 15 ± 3 | 14 ± 4 | 25 ± 3 | 128 ± 4 | 17 ± 3 |
| | 0 | 2.5 µl | 20 ± 4 | 18 ± 2* | 28 ± 2 | 118 ± 5 | 23 ± 1 |
| | 0 | 5 µl | 14 ± 2 | 12 ± 2 | 17 ± 2 | 129 ± 8 | 25 ± 1 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | 2.446 (2.527) | 3.852 (2.560) | -7.898 (4.122) | -15.737 (5.559) | -5.593 (5.609) |
| Y intercept (Standard error) | | | 14.421 (0.996) | 12.101 (1.009) | 26.617 (1.625) | 134.598 (2.207) | 25.746 (2.211) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 2 (concluded)**
**STATISTICAL ANALYSIS OF THE FIRST MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS**
**Experiment Performed: 1 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Tallow (UOP) | 5 | 0 µl | 16 ± 3 | 13 ± 2 | 40 ± 5 | 157 ± 4 | 31 ± 2 |
| | 5 | 0.156 µl | 16 ± 3 | 9 ± 1 | 30 ± 1 | 146 ± 4 | 30 ± 1 |
| | 5 | 0.313 µl | 34 ± 1* | 8 ± 2 | 29 ± 1 | 149 ± 6 | 35 ± 1 |
| | 5 | 0.625 µl | 22 ± 3 | 10 ± 2 | 35 ± 3 | 147 ± 8 | 28 ± 2 |
| | 5 | 1.25 µl | 20 ± 2 | 8 ± 2 | 34 ± 5 | 144 ± 3 | 19 ± 1 |
| | 5 | 2.5 µl | 16 ± 3 | 13 ± 2 | 27 ± 8 | 149 ± 2 | 30 ± 2 |
| | 5 | 5 µl | 16 ± 2 | 10 ± 2 | 31 ± 8 | 133 ± 6 | 28 ± 2 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | -6.942 (5.466) | 0.131 (2.044) | -6.346 (4.321) | -19.577 (5.031) | -5.598 (3.965) |
| Y intercept (Standard error) | | | 22.002 (2.115) | 10.119 (0.806) | 34.159 (1.703) | 152.351 (1.984) | 30.224 (1.563) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 3**
**STATISTICAL ANALYSIS OF THE SECOND MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 16 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Camelina (UOP) | 0 | 0 µl | 14 ± 2 | 9 ± 2 | 18 ± 3 | 117 ± 2 | 32 ± 2 |
| | 0 | 0.156 µl | 13 ± 3 | 14 ± 1 | 25 ± 5 | 135 ± 1* | 30 ± 2 |
| | 0 | 0.313 µl | 15 ± 2 | 13 ± 3 | 27 ± 3* | 120 ± 4 | 33 ± 4 |
| | 0 | 0.625 µl | 11 ± 1 | 12 ± 3 | 26 ± 1 | 109 ± 3 | 33 ± 5 |
| | 0 | 1.25 µl | 13 ± 1 | 15 ± 2 | 24 ± 4 | 98 ± 6 | 30 ± 5 |
| | 0 | 2.5 µl | 11 ± 3 | 15 ± 2 | 22 ± 2 | 85 ± 3 | 28 ± 2 |
| | 0 | 5 µl | 13 ± 2 | 11 ± 4 | 19 ± 4 | 82 ± 2 | 26 ± 4 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | -1.373 (2.030) | 1.654 (2.570) | -4.543 (3.507) | -62.428 (6.789) | -7.408 (2.695) |
| Y intercept (Standard error) | | | 13.024 (0.800) | 12.274 (1.013) | 24.198 (1.382) | 125.001 (2.676) | 32.330 (1.063) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 3 (continued)**
**STATISTICAL ANALYSIS OF THE SECOND MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 16 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Camelina (UOP) | 10 | 0 µl | 16 ± 2 | 13 ± 1 | 30 ± 3 | 125 ± 6 | 39 ± 3 |
| | 10 | 0.156 µl | 10 ± 2 | 13 ± 3 | 32 ± 3 | 114 ± 11 | 32 ± 5 |
| | 10 | 0.313 µl | 11 ± 1 | 12 ± 3 | 40 ± 6 | 118 ± 3 | 33 ± 6 |
| | 10 | 0.625 µl | 6 ± 2 | 12 ± 3 | 31 ± 6 | 114 ± 10 | 32 ± 4 |
| | 10 | 1.25 µl | 8 ± 2 | 20 ± 3 | 28 ± 5 | 110 ± 8 | 30 ± 1 |
| | 10 | 2.5 µl | 11 ± 1 | 13 ± 3 | 30 ± 0 | 108 ± 4 | 32 ± 4 |
| | 10 | 5 µl | 13 ± 3 | 0 ± 2 | 34 ± 3 | 99 ± 7 | 36 ± 4 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | -0.002 (2.942) | -1.493 (3.014) | -1.177 (4.214) | -27.771 (5.866) | -0.768 (3.657) |
| Y intercept (Standard error) | | | 10.763 (1.160) | 13.774 (1.188) | 32.443 (1.661) | 120.771 (2.313) | 33.703 (1.442) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 3 (continued)**
**STATISTICAL ANALYSIS OF THE SECOND MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 16 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Tallow (UOP) | 0 | 0 µl | 14 ± 2 | 9 ± 2 | 18 ± 3 | 117 ± 2 | 32 ± 2 |
| | 0 | 0.156 µl | 11 ± 2 | 8 ± 3 | 20 ± 2 | 139 ± 5* | 24 ± 2 |
| | 0 | 0.313 µl | 8 ± 3 | 7 ± 5 | 21 ± 4 | 127 ± 6 | 23 ± 2 |
| | 0 | 0.625 µl | 9 ± 2 | 10 ± 2 | 16 ± 5 | 130 ± 8 | 21 ± 2 |
| | 0 | 1.25 µl | 6 ± 1 | 7 ± 3 | 18 ± 6 | 115 ± 4 | 25 ± 4 |
| | 0 | 2.5 µl | 7 ± 2 | 8 ± 2 | 21 ± 6 | 123 ± 8 | 28 ± 4 |
| | 0 | 5 µl | 7 ± 1 | 6 ± 2 | 15 ± 1 | 134 ± 8 | 27 ± 1 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | N | N |
| Slope (Standard error) | | | -6.742 (2.015) | -2.751 (2.120) | -2.637 (3.380) | 2.557 (8.447) | 1.967 (3.463) |
| Y intercept (Standard error) | | | 10.752 (0.794) | 8.622 (0.836) | 19.302 (1.333) | 125.626 (3.330) | 25.134 (1.365) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant

**Table 3 (concluded)**
**STATISTICAL ANALYSIS OF THE SECOND MUTAGENICITY EXPERIMENT**
**WITH TWO HRJ FUELS**
**Experiment Performed: 16 March 2011**

| Test Article | S9 (%) | Dose/ Plate | Mean ± Standard Deviation Revertants/Plate TA1535 | TA1537 | TA98 | TA100 | WP2*uvr*A |
|---|---|---|---|---|---|---|---|
| HRJ Tallow (UOP) | 10 | 0 µl | 16 ± 2 | 13 ± 1 | 30 ± 3 | 125 ± 6 | 39 ± 3 |
| | 10 | 0.156 µl | 11 ± 2 | 12 ± 2 | 32 ± 2 | 154 ± 2* | 25 ± 2 |
| | 10 | 0.313 µl | 14 ± 3 | 11 ± 4 | 27 ± 4 | 146 ± 1* | 32 ± 2 |
| | 10 | 0.625 µl | 13 ± 1 | 10 ± 0 | 31 ± 2 | 156 ± 8* | 32 ± 5 |
| | 10 | 1.25 µl | 15 ± 2 | 12 ± 3 | 26 ± 2 | 155 ± 7* | 29 ± 3 |
| | 10 | 2.5 µl | 14 ± 2 | 10 ± 4 | 29 ± 3 | 159 ± 9* | 28 ± 3 |
| | 10 | 5 µl | 15 ± 2 | 10 ± 3 | 30 ± 2 | 156 ± 5* | 28 ± 1 |
| **ANALYSIS SUMMARY** | | | | | | | |
| Levene's test | | | N | N | N | N | N |
| Dose response evaluated via log log regression | | | N | N | N | S | N |
| Slope (Standard error) | | | 1.593 (1.886) | -2.558 (2.101) | -1.138 (2.336) | 25.248 (8.638) | -7.218 (3.738) |
| Y intercept (Standard error) | | | 13.672 (0.744) | 11.859 (0.828) | 29.479 (0.921) | 142.689 (3.405) | 32.607 (1.473) |

* = Significant ($p < 0.01$) by Dunnett's Test
N = Not significant
S = Significant ($p < 0.01$) by specified analyses

## Table 4
## INDIVIDUAL AND MEAN PLATE COUNTS
## 1st MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-11 Positive Controls**
**Experiment: G371-11 1st Mutagenicity**
Assay Conditions: Plate incorporation assay

Study Code: G371-11
Date Plated: 3/1/2011
Date Counted: 3/4/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **SA** | 5 µl | *1804.3* | *27.6* | *123.0* | 1802, 1778, 1833 |
| **TA1537** | **9AA** | 50 µl | *584.3* | *50.8* | *51.6* | 643, 554, 556 |
| **TA98** | **2NF** | 5 µl | *1537.3* | *15.9* | *48.0* | 1519, 1548, 1545 |
| **TA100** | **SA** | 5 µl | *2258.0* | *57.7* | *15.9* | 2217, 2324, 2233 |
| **WP2uvrA** | **4NQO** | 2.5 µl | *3291.3* | *45.6* | *85.9* | 3265, 3344, 3265 |
| **TA1535** | **DMSO** | -- | *11.0* | *1.0* | *0.8* | 10, 12, 11 |
| **TA1537** | **DMSO** | -- | *15.7* | *2.1* | *1.4* | 18, 14, 15 |
| **TA98** | **DMSO** | -- | *16.7* | *3.1* | *0.5* | 14, 16, 20 |
| **TA100** | **DMSO** | -- | *138.3* | *2.5* | *1.0* | 141, 136, 138 |
| **WP2uvrA** | **DMSO** | -- | *27.3* | *2.5* | *0.7* | 25, 30, 27 |

**With metabolic activation (5% S9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **2AN (5% S9)** | 4 µl | *246.3* | *24.5* | *15.7* | 222, 271, 246 |
| **TA1537** | **2AN (5% S9)** | 4 µl | *667.0* | *28.0* | *50.0* | 655, 699, 647 |
| **TA98** | **2AN (5% S9)** | 2 µl | *1982.0* | *180.4* | *50.0* | 2172, 1961, 1813 |
| **TA100** | **2AN (5% S9)** | 2 µl | *2879.0* | *79.6* | *18.3* | 2956, 2884, 2797 |
| **WP2uvrA** | **2AN (5% S9)** | 20 µl | *723.7* | *12.0* | *23.3* | 736, 712, 723 |
| **TA1535** | **DMSO (+S9)** | -- | *16.3* | *2.5* | *1.0* | 14, 16, 19 |
| **TA1537** | **DMSO (+S9)** | -- | *16.0* | *2.6* | *1.2* | 15, 19, 14 |
| **TA98** | **DMSO (+S9)** | -- | *33.3* | *1.5* | *0.8* | 33, 35, 32 |
| **TA100** | **DMSO (+S9)** | -- | *152.0* | *8.9* | *1.0* | 149, 145, 162 |
| **WP2uvrA** | **DMSO (+S9)** | -- | *37.3* | *4.0* | *1.2* | 35, 42, 35 |

Key to Positive Controls

| | |
|---|---|
| SA | Sodium Azide |
| 9AA | 9-Aminoacridine hydrochloride |
| 2NF | 2-Nitrofluorene |
| 4NQO | 4-Nitroquinoline N-oxide |
| DMSO | Dimethyl Sulfoxide |
| 2AN (5% S9) | 2-Aminoanthracene (5% S9) |
| DMSO (+S9) | Dimethyl Sulfoxide +S9 |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background lawn |

**Table 4 (continued)**
**INDIVIDUAL AND MEAN PLATE COUNTS**
**1st MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS**

**Study Name: G371-11 Camelina** Study Code: G371-11 Camelina
**Experiment: G371-11 1st Mutagenicity** Date Plated: 3/1/2011
Assay Conditions: Plate incorporation assay Date Counted: 3/4/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Camelina** | 0.156 µl | *21.3* | *3.2* | *1.5* | 25, 19, 20 |
| | | 0.313 µl | *23.0* | *3.6* | *1.6* | 20, 22, 27 |
| | | 0.625 µl | *14.3* | *2.1* | *1.0* | 12, 15, 16 |
| | | 1.25 µl | *18.7* | *4.2* | *1.3* | 22, 20, 14 |
| | | 2.5 µl | *12.7* | *2.1* | *0.9* | 15 N, 11 N, 12 N |
| | | 5 µl | *13.0* | *2.6* | *0.9* | 16 N, 11 N, 12 N |
| | **DMSO** | | *14.7* | *2.5* | | 15, 17, 12 |
| | **Untreated Control** | | *16.0* | *3.4* | | 15, 14, 19, 12, 20 |
| **TA1537** | **HRJ Camelin** | 0.156 µl | *13.0* | *4.4* | *1.1* | 10, 11, 18 |
| | | 0.313 µl | *14.0* | *2.0* | *1.2* | 12, 14, 16 |
| | | 0.625 µl | *12.3* | *1.5* | *1.1* | 11, 14, 12 |
| | | 1.25 µl | *12.0* | *3.5* | *1.1* | 10, 16, 10 |
| | | 2.5 µl | *11.0* | *3.6* | *1.0* | 10 N, 15 N, 8 N |
| | | 5 µl | *11.0* | *1.0* | *1.0* | 11 N, 12 N, 10 N |
| | **DMSO** | | *11.3* | *0.6* | | 12, 11, 11 |
| | **Untreated Control** | | *12.6* | *3.0* | | 12, 18, 11, 11, 11 |
| **TA98** | **HRJ Camelina** | 0.156 µl | *37.7* | *3.2* | *1.2* | 34, 40, 39 |
| | | 0.313 µl | *27.3* | *2.3* | *0.9* | 26, 30, 26 |
| | | 0.625 µl | *22.7* | *6.4* | *0.7* | 30, 20, 18 |
| | | 1.25 µl | *24.3* | *4.0* | *0.8* | 25, 28, 20 |
| | | 2.5 µl | *24.0* | *4.6* | *0.8* | 29 N, 23 N, 20 N |
| | | 5 µl | *27.3* | *3.8* | *0.9* | 29 N, 23 N, 30 N |
| | **DMSO** | | *32.0* | *1.7* | | 33, 33, 30 |
| | **Untreated Control** | | *29.4* | *4.3* | | 25, 35, 31, 31, 25 |
| **TA100** | **HRJ Camelina** | 0.156 µl | *136.3* | *7.5* | *1.0* | 132, 132, 145 |
| | | 0.313 µl | *145.0* | *9.0* | *1.0* | 154, 145, 136 |
| | | 0.625 µl | *112.0* | *7.5* | *0.8* | 113, 104, 119 |
| | | 1.25 µl | *124.0* | *10.4* | *0.9* | 119, 117, 136 |
| | | 2.5 µl | *145.0* | *7.2* | *1.0* | 139 N, 143 N, 153 N |
| | | 5 µl | *118.0* | *7.9* | *0.8* | 124 N, 121 N, 109 N |
| | **DMSO** | | *141.7* | *0.6* | | 141, 142, 142 |
| | **Untreated Control** | | *128.2* | *8.9* | | 121, 141, 128, 132, 119 |
| **WP2uvrA** | **HRJ Camelina** | 0.156 µl | *23.7* | *3.5* | *0.6* | 27, 20, 24 |
| | | 0.313 µl | *18.3* | *3.5* | *0.5* | 18, 22, 15 |
| | | 0.625 µl | *25.7* | *2.3* | *0.7* | 27, 27, 23 |
| | | 1.25 µl | *27.0* | *4.4* | *0.7* | 30, 29, 22 |
| | | 2.5 µl | *23.7* | *2.1* | *0.6* | 26 N, 22 N, 23 N |
| | | 5 µl | *31.3* | *1.5* | *0.8* | 30 N, 31 N, 33 N |
| | **DMSO** | | *38.3* | *2.3* | | 41, 37, 37 |
| | **Untreated Control** | | *40.2* | *6.3* | | 38, 40, 46, 31, 46 |

Key to Plate Postfix Codes

N Normal background lawn

## Table 4 (continued)
## INDIVIDUAL AND MEAN PLATE COUNTS
## 1st MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-11 Camelina**
**Experiment: G371-11 1st Mutagenicity**
Assay Conditions: Plate incorporation assay

Study Code: G371-11
Date Plated: 3/1/2011
Date Counted: 3/4/2011

**With metabolic activation (5% S9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Camelina** | 0.156 µl | *14.3* | *2.1* | *0.9* | 12, 16, 15 |
| | | 0.313 µl | *12.0* | *0.0* | *0.8* | 12, 12, 12 |
| | | 0.625 µl | *15.3* | *3.5* | *1.0* | 15, 12, 19 |
| | | 1.25 µl | *16.7* | *1.2* | *1.1* | 16, 16, 18 |
| | | 2.5 µl | *10.0* | *1.7* | *0.6* | 11 N, 8 N, 11 N |
| | | 5 µl | *15.7* | *3.1* | *1.0* | 15 N, 19 N, 13 N |
| | **DMSO** | | *15.7* | *3.2* | | 17, 18, 12 |
| **TA1537** | **HRJ Camelina** | 0.156 µl | *11.0* | *1.0* | *0.8* | 10, 11, 12 |
| | | 0.313 µl | *12.3* | *3.2* | *0.9* | 10, 11, 16 |
| | | 0.625 µl | *12.3* | *1.5* | *0.9* | 14, 11, 12 |
| | | 1.25 µl | *11.3* | *0.6* | *0.9* | 11, 11, 12 |
| | | 2.5 µl | *12.7* | *2.1* | *1.0* | 15 N, 12 N, 11 N |
| | | 5 µl | *10.0* | *0.0* | *0.8* | 10 N, 10 N, 10 N |
| | **DMSO** | | *13.3* | *2.1* | | 14, 15, 11 |
| **TA98** | **HRJ Camelina** | 0.156 µl | *42.3* | *4.7* | *1.1* | 46, 44, 37 |
| | | 0.313 µl | *34.0* | *5.2* | *0.9* | 40, 31, 31 |
| | | 0.625 µl | *36.3* | *6.0* | *0.9* | 30, 42, 37 |
| | | 1.25 µl | *28.7* | *1.5* | *0.7* | 30, 27, 29 |
| | | 2.5 µl | *30.3* | *4.2* | *0.8* | 27 N, 35 N, 29 N |
| | | 5 µl | *34.3* | *5.1* | *0.9* | 40 N, 33 N, 30 N |
| | **DMSO** | | *39.7* | *5.1* | | 44, 34, 41 |
| **TA100** | **HRJ Camelina** | 0.156 µl | *133.0* | *2.6* | *0.8* | 131, 132, 136 |
| | | 0.313 µl | *127.3* | *16.8* | *0.8* | 108, 138, 136 |
| | | 0.625 µl | *139.3* | *5.8* | *0.9* | 136, 146, 136 |
| | | 1.25 µl | *147.3* | *12.7* | *0.9* | 139, 141, 162 |
| | | 2.5 µl | *150.3* | *5.5* | *1.0* | 150 N, 156 N, 145 N |
| | | 5 µl | *142.7* | *9.0* | *0.9* | 152 N, 134 N, 142 N |
| | **DMSO** | | *157.0* | *4.0* | | 153, 157, 161 |
| **WP2uvrA** | **HRJ Camelina** | 0.156 µl | *29.0* | *4.0* | *0.9* | 33, 29, 25 |
| | | 0.313 µl | *37.0* | *4.4* | *1.2* | 34, 42, 35 |
| | | 0.625 µl | *36.0* | *5.2* | *1.2* | 33, 42, 33 |
| | | 1.25 µl | *26.7* | *4.0* | *0.9* | 29, 22, 29 |
| | | 2.5 µl | *33.0* | *5.3* | *1.1* | 37 N, 35 N, 27 N |
| | | 5 µl | *38.3* | *6.7* | *1.2* | 31 N, 44 N, 40 N |
| | **DMSO** | | *31.0* | *2.0* | | 33, 29, 31 |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background lawn |

**Table 4 (continued)**
**INDIVIDUAL AND MEAN PLATE COUNTS**
**1st MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS**

**Study Name: G371-11 Tallow** Study Code: G371-11
**Experiment: G371-11 1st Mutagenicity** Date Plated: 3/1/2011
Assay Conditions: Plate incorporation assay Date Counted: 3/4/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Tallow** | 0.156 µl | *14.0* | *2.0* | *1.0* | 14, 12, 16 |
| | | 0.313 µl | *14.3* | *0.6* | *1.0* | 15, 14, 14 |
| | | 0.625 µl | *14.3* | *2.1* | *1.0* | 15, 16, 12 |
| | | 1.25 µl | *14.7* | *3.1* | *1.0* | 12, 18, 14 |
| | | 2.5 µl | *20.3* | *4.0* | *1.4* | 18 N, 25 N, 18 N |
| | | 5 µl | *13.7* | *1.5* | *0.9* | 15 N, 12 N, 14 N |
| | **DMSO** | | *14.7* | *2.5* | | 15, 17, 12 |
| | **Untreated Control** | | *16.0* | *3.4* | | 15, 14, 19, 12, 20 |
| **TA1537** | **HRJ Tallow** | 0.156 µl | *11.7* | *0.6* | *1.0* | 12, 11, 12 |
| | | 0.313 µl | *14.7* | *1.2* | *1.3* | 14, 14, 16 |
| | | 0.625 µl | *10.0* | *2.0* | *0.9* | 12, 10, 8 |
| | | 1.25 µl | *14.3* | *4.0* | *1.3* | 12, 19, 12 |
| | | 2.5 µl | *18.3* | *1.5* | *1.6* | 20 N, 17 N, 18 N |
| | | 5 µl | *12.3* | *2.3* | *1.1* | 11 N, 15 N, 11 N |
| | **DMSO** | | *11.3* | *0.6* | | 12, 11, 11 |
| | **Untreated Control** | | *12.6* | *3.0* | | 12, 18, 11, 11, 11 |
| **TA98** | **HRJ Tallow** | 0.156 µl | *24.0* | *5.2* | *0.8* | 18, 27, 27 |
| | | 0.313 µl | *21.0* | *4.6* | *0.7* | 22, 25, 16 |
| | | 0.625 µl | *22.3* | *0.6* | *0.7* | 22, 23, 22 |
| | | 1.25 µl | *25.0* | *2.6* | *0.8* | 22, 26, 27 |
| | | 2.5 µl | *28.3* | *2.1* | *0.9* | 29 N, 30 N, 26 N |
| | | 5 µl | *17.3* | *2.1* | *0.5* | 15 N, 19 N, 18 N |
| | **DMSO** | | *32.0* | *1.7* | | 33, 33, 30 |
| | **Untreated Control** | | *29.4* | *4.3* | | 25, 35, 31, 31, 25 |
| **TA100** | **HRJ Tallow** | 0.156 µl | *129.7* | *3.8* | *0.9* | 134, 127, 128 |
| | | 0.313 µl | *134.3* | *3.5* | *0.9* | 131, 134, 138 |
| | | 0.625 µl | *128.7* | *4.9* | *0.9* | 132, 131, 123 |
| | | 1.25 µl | *128.0* | *4.4* | *0.9* | 131, 123, 130 |
| | | 2.5 µl | *118.3* | *4.9* | *0.8* | 124 N, 116 N, 115 N |
| | | 5 µl | *129.0* | *7.9* | *0.9* | 138 N, 123 N, 126 N |
| | **DMSO** | | *141.7* | *0.6* | | 141, 142, 142 |
| | **Untreated Control** | | *128.2* | *8.9* | | 121, 141, 128, 132, 119 |
| **WP2uvrA** | **HRJ Tallow** | 0.156 µl | *19.0* | *1.0* | *0.5* | 20, 18, 19 |
| | | 0.313 µl | *22.0* | *3.5* | *0.6* | 26, 20, 20 |
| | | 0.625 µl | *23.7* | *1.2* | *0.6* | 25, 23, 23 |
| | | 1.25 µl | *17.3* | *2.5* | *0.5* | 20, 17, 15 |
| | | 2.5 µl | *23.0* | *1.0* | *0.6* | 23 N, 24 N, 22 N |
| | | 5 µl | *25.3* | *1.2* | *0.7* | 24 N, 26 N, 26 N |
| | **DMSO** | | *38.3* | *2.3* | | 41, 37, 37 |
| | **Untreated Control** | | *40.2* | *6.3* | | 38, 40, 46, 31, 46 |

Key to Plate Postfix Codes

N Normal background lawn

## Table 4 (concluded)
## INDIVIDUAL AND MEAN PLATE COUNTS
## 1st MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-11 Tallow**
**Experiment: G371-11 1st Mutagenicity**
Assay Conditions: Plate incorporation assay

Study Code: G371-11
Date Plated: 3/1/2011
Date Counted: 3/4/2011

**With metabolic activation (5% S9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Tallow** | 0.156 µl | *16.0* | *2.6* | *1.0* | 19, 14, 15 |
| | | 0.313 µl | *34.0* | *1.0* | *2.2* | 35, 34, 33 |
| | | 0.625 µl | *21.7* | *2.9* | *1.4* | 20, 25, 20 |
| | | 1.25 µl | *20.3* | *2.3* | *1.3* | 19, 23, 19 |
| | | 2.5 µl | *16.0* | *2.6* | *1.0* | 15 N, 14 N, 19 N |
| | | 5 µl | *16.0* | *2.0* | *1.0* | 18 N, 16 N, 14 N |
| | **DMSO** | | *15.7* | *3.2* | | 17, 18, 12 |
| **TA1537** | **HRJ Tallow** | 0.156 µl | *9.3* | *1.2* | *0.7* | 10, 8, 10 |
| | | 0.313 µl | *8.3* | *1.5* | *0.6* | 10, 8, 7 |
| | | 0.625 µl | *9.7* | *1.5* | *0.7* | 10, 8, 11 |
| | | 1.25 µl | *8.3* | *1.5* | *0.6* | 10, 8, 7 |
| | | 2.5 µl | *12.7* | *2.1* | *1.0* | 11 N, 12 N, 15 N |
| | | 5 µl | *10.0* | *2.0* | *0.8* | 10 N, 12 N, 8 N |
| | **DMSO** | | *13.3* | *2.1* | | 14, 15, 11 |
| **TA98** | **HRJ Tallow** | 0.156 µl | *29.7* | *1.2* | *0.7* | 29, 29, 31 |
| | | 0.313 µl | *29.3* | *0.6* | *0.7* | 29, 30, 29 |
| | | 0.625 µl | *35.0* | *2.6* | *0.9* | 38, 33, 34 |
| | | 1.25 µl | *37.3* | *0.6* | *0.9* | 37, 37, 38 |
| | | 2.5 µl | *30.7* | *2.9* | *0.8* | 34 N, 29 N, 29 N |
| | | 5 µl | *31.0* | *1.7* | *0.8* | 33 N, 30 N, 30 N |
| | **DMSO** | | *39.7* | *5.1* | | 44, 34, 41 |
| **TA100** | **HRJ Tallow** | 0.156 µl | *146.0* | *3.6* | *0.9* | 145, 150, 143 |
| | | 0.313 µl | *149.0* | *6.1* | *0.9* | 146, 156, 145 |
| | | 0.625 µl | *147.3* | *7.5* | *0.9* | 156, 143, 143 |
| | | 1.25 µl | *144.3* | *3.1* | *0.9* | 141, 145, 147 |
| | | 2.5 µl | *149.0* | *1.7* | *0.9* | 150 N, 150 N, 147 N |
| | | 5 µl | *133.3* | *5.7* | *0.8* | 127 N, 138 N, 135 N |
| | **DMSO** | | *157.0* | *4.0* | | 153, 157, 161 |
| **WP2uvrA** | **HRJ Tallow** | 0.156 µl | *29.7* | *0.6* | *1.0* | 30, 29, 30 |
| | | 0.313 µl | *34.7* | *0.6* | *1.1* | 35, 34, 35 |
| | | 0.625 µl | *28.3* | *2.3* | *0.9* | 31, 27, 27 |
| | | 1.25 µl | *18.7* | *1.2* | *0.6* | 20, 18, 18 |
| | | 2.5 µl | *30.0* | *1.7* | *1.0* | 31 N, 31 N, 28 N |
| | | 5 µl | *27.7* | *2.1* | *0.9* | 27 N, 30 N, 26 N |
| | **DMSO** | | *31.0* | *2.0* | | 33, 29, 31 |

Key to Plate Postfix Codes

N Normal background lawn

## Table 5
## INDIVIDUAL AND MEAN PLATE COUNTS
## 2nd MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-11 Positive Controls**
**Experiment: G371-11 2nd Mutagenicity**
Assay Conditions: Plate incorporation assay

Study Code: G371-1
Date Plated: 3/16/2011
Date Counted: 3/18/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **SA** | 5 µg | *927.0* | *87.2* | *67.8* | 987, 827, 967 |
| **TA1537** | **9AA** | 50 µg | *238.3* | *28.7* | *26.5* | 242, 208, 265 |
| **TA98** | **2NF** | 5 µg | *759.0* | *41.7* | *43.0* | 780, 786, 711 |
| **TA100** | **SA** | 5 µg | *803.0* | *15.9* | *6.8* | 791, 797, 821 |
| **WP2uvrA** | **4NQO** | 2.5 µg | *3638.7* | *195.6* | *114.9* | 3854, 3590, 3472 |
| **TA1535** | **DMSO** | -- | *7.0* | *1.0* | *0.5* | 6, 7, 8 |
| **TA1537** | **DMSO** | -- | *10.3* | *0.6* | *1.1* | 10, 10, 11 |
| **TA98** | **DMSO** | -- | *12.7* | *3.1* | *0.7* | 12, 16, 10 |
| **TA100** | **DMSO** | -- | *109.7* | *7.6* | *0.9* | 103, 108, 118 |
| **WP2uvrA** | **DMSO** | -- | *24.7* | *2.5* | *0.8* | 22, 25, 27 |

**With metabolic activation (10% S9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **2AN (10% S9)** | 4 µg | *194.3* | *10.4* | *12.4* | 191, 186, 206 |
| **TA1537** | **2AN (10% S9)** | 4 µg | *457.3* | *49.1* | *36.1* | 420, 439, 513 |
| **TA98** | **2AN (10% S9)** | 2 µg | *849.3* | *35.9* | *28.6* | 873, 808, 867 |
| **TA100** | **2AN (10% S9)** | 2 µg | *1063.7* | *58.3* | *8.5* | 1131, 1028, 1032 |
| **WP2uvrA** | **2AN (10% S9)** | 20 µg | *320.7* | *6.8* | *8.3* | 326, 323, 313 |
| **TA1535** | **DMSO (+S9)** | -- | *9.3* | *2.1* | *0.6* | 11, 7, 10 |
| **TA1537** | **DMSO (+S9)** | -- | *14.3* | *3.2* | *1.1* | 18, 13, 12 |
| **TA98** | **DMSO (+S9)** | -- | *32.3* | *4.6* | *1.1* | 27, 35, 35 |
| **TA100** | **DMSO (+S9)** | -- | *129.0* | *0.0* | *1.0* | 129, 129, 129 |
| **WP2uvrA** | **DMSO (+S9)** | -- | *34.7* | *2.1* | *0.9* | 33, 37, 34 |

| Key to Positive Controls | |
|---|---|
| SA | Sodium Azide |
| 9AA | 9-Aminoacridine hydrochloride |
| 2NF | 2-Nitrofluorene |
| 4NQO | 4-Nitroquinoline N-oxide |
| DMSO | Dimethyl Sulfoxide |
| 2AN (10% S9) | 2-Aminoanthracene (10% S9) |
| DMSO (+S9) | Dimethyl Sulfoxide +S9 |

| Key to Plate Postfix Codes | |
|---|---|
| N | Normal background lawn |

**Table 5 (continued)**
**INDIVIDUAL AND MEAN PLATE COUNTS**
**2nd MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS**

**Study Name: G371-11 Camelina** Study Code: G371-11
**Experiment: G371-11 2nd Mutagenicity** Date Plated: 3/16/2011
Assay Conditions: Plate incorporation assay Date Counted: 3/18/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Camelina** | 0.156 µl | *12.7* | *2.9* | *0.9* | 11, 11, 16 |
| | | 0.313 µl | *14.7* | *3.1* | *1.1* | 12, 18, 14 |
| | | 0.625 µl | *10.7* | *1.2* | *0.8* | 12, 10, 10 |
| | | 1.25 µl | *12.7* | *1.2* | *0.9* | 14, 12, 12 |
| | | 2.5 µl | *10.7* | *3.1* | *0.8* | 14, 10, 8 |
| | | 5 µl | *13.3* | *2.3* | *1.0* | 12 N, 12 N, 16 N |
| | **DMSO** | | *13.7* | *1.5* | | 12, 14, 15 |
| | **Untreated Control** | | *5.4* | *0.9* | | 5, 5, 7, 5, 5 |
| **TA1537** | **HRJ Camelina** | 0.156 µl | *14.3* | *0.6* | *1.6* | 14, 14, 15 |
| | | 0.313 µl | *11.7* | *1.5* | *1.3* | 10, 13, 12 |
| | | 0.625 µl | *11.7* | *2.9* | *1.3* | 10, 15, 10 |
| | | 1.25 µl | *15.3* | *2.1* | *1.7* | 13, 17, 16 |
| | | 2.5 µl | *15.3* | *1.5* | *1.7* | 14, 17, 15 |
| | | 5 µl | *11.0* | *3.6* | *1.2* | 15 N, 10 N, 8 N |
| | **DMSO** | | *9.0* | *1.7* | | 10, 7, 10 |
| | **Untreated Control** | | *9.2* | *3.1* | | 12, 5, 10, 7, 12 |
| **TA98** | **HRJ Camelina** | 0.156 µl | *25.0* | *4.6* | *1.4* | 20, 26, 29 |
| | | 0.313 µl | *27.0* | *2.6* | *1.5* | 30, 26, 25 |
| | | 0.625 µl | *25.7* | *0.6* | *1.5* | 26, 26, 25 |
| | | 1.25 µl | *21.0* | *3.6* | *1.2* | 20, 18, 25 |
| | | 2.5 µl | *21.7* | *1.5* | *1.2* | 22, 20, 23 |
| | | 5 µl | *18.7* | *3.5* | *1.1* | 22 N, 19 N, 15 N |
| | **DMSO** | | *17.7* | *2.5* | | 15, 20, 18 |
| | **Untreated Control** | | *34.0* | *0.7* | | 35, 34, 33, 34, 34 |
| **TA100** | **HRJ Camelina** | 0.156 µl | *135.0* | *1.0* | *1.2* | 136, 134, 135 |
| | | 0.313 µl | *119.7* | *4.0* | *1.0* | 119, 124, 116 |
| | | 0.625 µl | *108.7* | *3.1* | *0.9* | 108, 112, 106 |
| | | 1.25 µl | *98.0* | *6.2* | *0.8* | 105, 93, 96 |
| | | 2.5 µl | *88.3* | *3.2* | *0.8* | 87, 86, 92 |
| | | 5 µl | *82.3* | *2.3* | *0.7* | 85 N, 81 N, 81 N |
| | **DMSO** | | *117.3* | *2.1* | | 119, 115, 118 |
| | **Untreated Control** | | *96.8* | *6.0* | | 91, 105, 101, 92, 95 |
| **WP2uvrA** | **HRJ Camelina** | 0.156 µl | *30.0* | *1.7* | *0.9* | 31, 28, 31 |
| | | 0.313 µl | *32.7* | *3.8* | *1.0* | 31, 37, 30 |
| | | 0.625 µl | *32.7* | *4.6* | *1.0* | 38, 30, 30 |
| | | 1.25 µl | *30.0* | *4.6* | *0.9* | 29, 26, 35 |
| | | 2.5 µl | *27.3* | *1.5* | *0.9* | 29, 27, 26 |
| | | 5 µl | *26.3* | *4.2* | *0.8* | 31 N, 23 N, 25 N |
| | **DMSO** | | *31.7* | *2.3* | | 33, 29, 33 |
| | **Untreated Control** | | *29.8* | *5.0* | | 22, 33, 30, 29, 35 |

Key to Plate Postfix Codes

N Normal background lawn

**Table 5 (continued)**
**INDIVIDUAL AND MEAN PLATE COUNTS**
**2nd MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS**

**Study Name: G371-11 Camelina** Study Code: G371-11 Camelina
**Experiment: G371-11 2nd Mutagenicity** Date Plated: 3/16/2011
Assay Conditions: Plate incorporation assay Date Counted: 3/18/2011

**With metabolic activation (10% S9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Camelina** | 0.156 µl | *10.3* | *2.1* | *0.7* | 12, 8, 11 |
| | | 0.313 µl | *11.3* | *1.2* | *0.7* | 12, 10, 12 |
| | | 0.625 µl | *6.0* | *1.7* | *0.4* | 7, 7, 4 |
| | | 1.25 µl | *7.7* | *2.1* | *0.5* | 6, 7, 10 |
| | | 2.5 µl | *11.3* | *0.6* | *0.7* | 12, 11, 11 |
| | | 5 µl | *13.0* | *2.6* | *0.8* | 15 N, 10 N, 14 N |
| | **DMSO** | | *15.7* | *2.1* | | 14, 18, 15 |
| **TA1537** | **HRJ Camelina** | 0.156 µl | *13.0* | *2.6* | *1.0* | 15, 10, 14 |
| | | 0.313 µl | *12.3* | *2.5* | *1.0* | 15, 12, 10 |
| | | 0.625 µl | *12.3* | *3.2* | *1.0* | 10, 16, 11 |
| | | 1.25 µl | *19.7* | *2.5* | *1.6* | 22, 17, 20 |
| | | 2.5 µl | *13.3* | *3.1* | *1.1* | 14, 16, 10 |
| | | 5 µl | *10.0* | *2.0* | *0.8* | 10 N, 12 N, 8 N |
| | **DMSO** | | *12.7* | *1.2* | | 12, 14, 12 |
| **TA98** | **HRJ Camelina** | 0.156 µl | *32.3* | *2.9* | *1.1* | 34, 29, 34 |
| | | 0.313 µl | *39.7* | *6.1* | *1.3* | 45, 41, 33 |
| | | 0.625 µl | *31.0* | *5.6* | *1.0* | 26, 30, 37 |
| | | 1.25 µl | *28.3* | *5.0* | *1.0* | 23, 29, 33 |
| | | 2.5 µl | *30.0* | *0.0* | *1.0* | 30, 30, 30 |
| | | 5 µl | *33.7* | *2.5* | *1.1* | 36 N, 34 N, 31 N |
| | **DMSO** | | *29.7* | *2.5* | | 30, 32, 27 |
| **TA100** | **HRJ Camelina** | 0.156 µl | *113.7* | *11.0* | *0.9* | 110, 105, 126 |
| | | 0.313 µl | *118.3* | *3.2* | *0.9* | 116, 122, 117 |
| | | 0.625 µl | *114.3* | *10.1* | *0.9* | 108, 109, 126 |
| | | 1.25 µl | *110.0* | *7.5* | *0.9* | 117, 111, 102 |
| | | 2.5 µl | *107.7* | *3.8* | *0.9* | 112, 106, 105 |
| | | 5 µl | *98.7* | *7.1* | *0.8* | 100 N, 105 N, 91 N |
| | **DMSO** | | *125.3* | *5.5* | | 125, 131, 120 |
| **WP2uvrA** | **HRJ Camelina** | 0.156 µl | *32.0* | *4.6* | *0.8* | 33, 36, 27 |
| | | 0.313 µl | *33.3* | *5.8* | *0.9* | 30, 30, 40 |
| | | 0.625 µl | *32.0* | *4.4* | *0.8* | 35, 27, 34 |
| | | 1.25 µl | *30.3* | *0.6* | *0.8* | 31, 30, 30 |
| | | 2.5 µl | *32.0* | *3.6* | *0.8* | 31, 29, 36 |
| | | 5 µl | *36.0* | *3.6* | *0.9* | 40 N, 35 N, 33 N |
| | **DMSO** | | *38.7* | *2.9* | | 37, 42, 37 |

Key to Plate Postfix Codes

N Normal background lawn

**Table 5 (continued)**
**INDIVIDUAL AND MEAN PLATE COUNTS**
**2nd MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS**

**Study Name: G371-11 Tallow** Study Code: G371-11
**Experiment: G371-11 2nd Mutagenicity** Date Plated: 3/16/2011
Assay Conditions: Plate incorporation assay Date Counted: 3/18/2011

**Without metabolic activation**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Tallow** | 0.156 µl | *10.7* | *2.3* | *0.8* | 12, 12, 8 |
| | | 0.313 µl | *8.0* | *3.0* | *0.6* | 11, 8, 5 |
| | | 0.625 µl | *9.0* | *1.7* | *0.7* | 11, 8, 8 |
| | | 1.25 µl | *6.0* | *1.0* | *0.4* | 6, 7, 5 |
| | | 2.5 µl | *7.0* | *1.7* | *0.5* | 8, 8, 5 |
| | | 5 µl | *7.0* | *1.0* | *0.5* | 7 N, 8 N, 6 N |
| | **DMSO** | | *13.7* | *1.5* | | 12, 14, 15 |
| | **Untreated Control** | | *5.4* | *0.9* | | 5, 5, 7, 5, 5 |
| **TA1537** | **HRJ Tallow** | 0.156 µl | *8.0* | *2.6* | *0.9* | 5, 9, 10 |
| | | 0.313 µl | *10.0* | *1.0* | *1.1* | 9, 11, 10 |
| | | 0.625 µl | *10.0* | *2.0* | *1.1* | 12, 8, 10 |
| | | 1.25 µl | *7.3* | *2.5* | *0.8* | 10, 7, 5 |
| | | 2.5 µl | *7.7* | *1.5* | *0.9* | 8, 6, 9 |
| | | 5 µl | *6.0* | *1.7* | *0.7* | 5 N, 5 N, 8 N |
| | **DMSO** | | *9.0* | *1.7* | | 10, 7, 10 |
| | **Untreated Control** | | *9.2* | *3.1* | | 12, 5, 10, 7, 12 |
| **TA98** | **HRJ Tallow** | 0.156 µl | *19.7* | *2.1* | *1.1* | 19, 18, 22 |
| | | 0.313 µl | *21.3* | *4.0* | *1.2* | 26, 19, 19 |
| | | 0.625 µl | *16.3* | *4.7* | *0.9* | 18, 11, 20 |
| | | 1.25 µl | *18.3* | *5.8* | *1.0* | 15, 15, 25 |
| | | 2.5 µl | *21.0* | *5.6* | *1.2* | 26, 22, 15 |
| | | 5 µl | *15.3* | *0.6* | *0.9* | 15 N, 16 N, 15 N |
| | **DMSO** | | *17.7* | *2.5* | | 15, 20, 18 |
| | **Untreated Control** | | *34.0* | *0.7* | | 35, 34, 33, 34, 34 |
| **TA100** | **HRJ Tallow** | 0.156 µl | *139.3* | *5.0* | *1.2* | 134, 144, 140 |
| | | 0.313 µl | *127.0* | *6.0* | *1.1* | 121, 127, 133 |
| | | 0.625 µl | *129.7* | *8.3* | *1.1* | 123, 127, 139 |
| | | 1.25 µl | *115.0* | *4.4* | *1.0* | 112, 113, 120 |
| | | 2.5 µl | *122.7* | *8.1* | *1.0* | 119, 117, 132 |
| | | 5 µl | *133.7* | *8.4* | *1.1* | 138 N, 124 N, 139 N |
| | **DMSO** | | *117.3* | *2.1* | | 119, 115, 118 |
| | **Untreated Control** | | *96.8* | *6.0* | | 91, 105, 101, 92, 95 |
| **WP2uvrA** | **HRJ Tallow** | 0.156 µl | *24.0* | *2.0* | *0.8* | 26, 22, 24 |
| | | 0.313 µl | *23.0* | *1.7* | *0.7* | 22, 22, 25 |
| | | 0.625 µl | *20.7* | *1.5* | *0.7* | 19, 21, 22 |
| | | 1.25 µl | *25.0* | *3.6* | *0.8* | 24, 22, 29 |
| | | 2.5 µl | *28.3* | *4.0* | *0.9* | 26, 26, 33 |
| | | 5 µl | *27.3* | *0.6* | *0.9* | 27 N, 28 N, 27 N |
| | **DMSO** | | *31.7* | *2.3* | | 33, 29, 33 |
| | **Untreated Control** | | *29.8* | *5.0* | | 22, 33, 30, 29, 35 |

Key to Plate Postfix Codes

N Normal background lawn

## Table 5 (concluded)
## INDIVIDUAL AND MEAN PLATE COUNTS
## 2nd MUTAGENICITY EXPERIMENT WITH TWO HRJ FUELS

**Study Name: G371-1 Tallow**
**Experiment: G371-11 2nd Mutagenicity**
Assay Conditions: Plate incorporation assay

Study Code: G371-11
Date Plated: 3/16/2011
Date Counted: 3/18/2011

**With metabolic activation (10% S9)**

| Strain | Compound | Dose level per plate | Mean revertants per plate | Standard Deviation | Ratio treated / solvent | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| **TA1535** | **HRJ Tallow** | 0.156 µl | *11.3* | *1.5* | *0.7* | 13, 11, 10 |
| | | 0.313 µl | *14.3* | *3.1* | *0.9* | 15, 17, 11 |
| | | 0.625 µl | *13.3* | *0.6* | *0.9* | 13, 13, 14 |
| | | 1.25 µl | *15.3* | *2.3* | *1.0* | 18, 14, 14 |
| | | 2.5 µl | *13.7* | *2.3* | *0.9* | 11, 15, 15 |
| | | 5 µl | *15.3* | *1.5* | *1.0* | 17 N, 14 N, 15 N |
| | **DMSO** | | *15.7* | *2.1* | | 14, 18, 15 |
| **TA1537** | **HRJ Tallow** | 0.156 µl | *12.0* | *2.0* | *0.9* | 10, 12, 14 |
| | | 0.313 µl | *11.0* | *3.6* | *0.9* | 10, 8, 15 |
| | | 0.625 µl | *10.0* | *0.0* | *0.8* | 10, 10, 10 |
| | | 1.25 µl | *12.0* | *3.5* | *0.9* | 14, 14, 8 |
| | | 2.5 µl | *9.7* | *3.8* | *0.8* | 14, 8, 7 |
| | | 5 µl | *10.3* | *2.9* | *0.8* | 12 N, 12 N, 7 N |
| | **DMSO** | | *12.7* | *1.2* | | 12, 14, 12 |
| **TA98** | **HRJ Tallow** | 0.156 µl | *31.7* | *2.1* | *1.1* | 34, 31, 30 |
| | | 0.313 µl | *27.0* | *3.6* | *0.9* | 24, 26, 31 |
| | | 0.625 µl | *31.0* | *1.7* | *1.0* | 30, 33, 30 |
| | | 1.25 µl | *26.3* | *1.5* | *0.9* | 25, 28, 26 |
| | | 2.5 µl | *28.7* | *2.5* | *1.0* | 26, 31, 29 |
| | | 5 µl | *29.7* | *2.3* | *1.0* | 31 N, 31 N, 27 N |
| | **DMSO** | | *29.7* | *2.5* | | 30, 32, 27 |
| **TA100** | **HRJ Tallow** | 0.156 µl | *154.0* | *1.7* | *1.2* | 152, 155, 155 |
| | | 0.313 µl | *145.7* | *0.6* | *1.2* | 146, 146, 145 |
| | | 0.625 µl | *156.3* | *7.8* | *1.2* | 150, 165, 154 |
| | | 1.25 µl | *154.7* | *7.1* | *1.2* | 147, 156, 161 |
| | | 2.5 µl | *158.7* | *8.6* | *1.3* | 168, 157, 151 |
| | | 5 µl | *156.3* | *4.9* | *1.2* | 153 N, 154 N, 162 N |
| | **DMSO** | | *125.3* | *5.5* | | 125, 131, 120 |
| **WP2uvrA** | **HRJ Tallow** | 0.156 µl | *25.0* | *2.0* | *0.6* | 27, 25, 23 |
| | | 0.313 µl | *32.0* | *1.7* | *0.8* | 33, 30, 33 |
| | | 0.625 µl | *32.3* | *4.6* | *0.8* | 27, 35, 35 |
| | | 1.25 µl | *29.3* | *2.9* | *0.8* | 31, 31, 26 |
| | | 2.5 µl | *28.0* | *2.6* | *0.7* | 29, 25, 30 |
| | | 5 µl | *28.0* | *1.0* | *0.7* | 29 N, 27 N, 28 N |
| | **DMSO** | | *38.7* | *2.9* | | 37, 42, 37 |

Key to Plate Postfix Codes

| | |
|---|---|
| N | Normal background lawn |

## APPENDIX H. HISTORICAL VALUES FOR SPONTANEOUS REVERTANTS AND POSITIVE CONTROLS

Historical data include GLP studies conducted at SRI International from 1/05 to 3/10.

| Strain | Spontaneous Revertants |
|---|---|
| TA1535 | 5 - 35 |
| TA1537 | 1 - 20 |
| TA98 | 10 - 45 |
| TA100 | 90 - 210 |
| WP2*uvr*A | 10 – 50 |

| Strain | Positive Control | S9 (%) | Dose/Plate | Range |
|---|---|---|---|---|
| TA1535 | sodium azide | 0 | 5 µg | 780 - 2680 |
| TA1537 | 9-aminoacridine | 0 | 50 µg | 108 - 800 |
| TA98 | 2-nitrofluorene | 0 | 5 µg | 640 - 2790 |
| TA100 | sodium azide | 0 | 5 µg | 860 - 2630 |
| WP2*uvr*A | 4-Nitroquinoline-N-oxide | 0 | 2.5 µg | 1285 – 4511 |
| TA1535 | 2-anthramine | 5/10 | 4 µg | 215-652/180-500 |
| TA1537 | 2-anthramine | 5/10 | 4 µg | 265-920/225-710 |
| TA98 | 2-anthramine | 5/10 | 2 µg | 865-3905/805-2790 |
| TA100 | 2-anthramine | 5/10 | 2 µg | 1065-4800/1005-3085 |
| WP2*uvr*A | 2-anthramine | 5/10 | 20 µg | 225-920/140-775 |

## LIST OF ACRONYMS

| | |
|---|---|
| °C | degrees Celsius |
| µg | microgram |
| µL | microliter |
| AAALAC | Association for Assessment and Accreditation of Laboratory Animal Care |
| AMS | Archive Management System |
| cm | centimeter |
| CoFA | Certificate of Analysis |
| DMSO | dimethyl sulfoxide |
| DTIC | Defense Technical Information Center |
| EPA | Environmental Protection Agency |
| F-T | Fischer-Tropsch |
| g | gram |
| GLP | Good Laboratory Practices |
| HEFA | hydroprocessed esters and fatty acids |
| HEFA-C | HEFA-Camelina |
| HEFA-F | HEFA-Animal fats and oils |
| HEFA-T | HEFA-Tallow |
| HJF | Henry M. Jackson Foundation for the Advancement of Military Medicine |
| HRJ | hydrotreated renewable jet |
| kg | kilogram |
| M | molar |
| MA | metabolic activation |
| mg | milligram |
| min | minutes |
| mL | milliliter |
| mm | millimiter |
| NCIMB | National Collection of Industrial and Marine Bacteria |
| NZW | New Zealand White |
| OECD | Organisation for Economic Cooperation and Development |
| OPPTS | Office of Prevention, Pesticides and Toxic Substances |
| PDII | Primary Dermal Irritation Index |
| rpm | rotations per minute |
| sec | seconds |
| SOP | standard operating procedure |
| UV | ultraviolet |
| v/v | volume/volume |
| WIL Research | WIL Research Laboratories, LLC |