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**Acute Dermal Irritation Study of JP-8 and S-8 in  
New Zealand White Rabbits**

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| <b>14. ABSTRACT</b><br>The objective of this study was to determine the irritative potential of JP-8, S-8 and a 50/50 volume/volume mixture of JP-8 and S-8 following a single exposure to the skin of albino rabbits. Three groups of three albino rabbits received a single, four-hour, occluded exposure and an additional three groups received a single, four-hour, semi-occluded exposure. Doses (0.5 mL) of the test substances were applied to clipped, unabraded skin. At the completion of all exposures, the bandages were removed and the sites washed. Application sites were evaluated at approximately 30 to 60 minutes; 24, 48 and 72 hours; and 4, 7 and 14 days after patch removal for sites where irritation persisted. Occluded exposures of JP-8 or S-8 resulted in a classification of moderately irritating. Semi-occlusion of either JP-8 or S-8 produced slight irritation. The mixture, however, was slightly irritating in both the occluded and semi-occluded exposures. |                    |                                   |                                  |                                                                                      |  |
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## **PREFACE**

Funding for this project was provided through the Air Force Research Laboratory, Propulsion Directorate, Fuels Branch (Dr Tim Edwards, AFRL/RZPF) and the Alternative Fuels Certification Office (AFMC 77 AESW/LF). This research was conducted under contract FA8601-07-P-0241. The program manager for the contract was LT Dean Wagner, PhD, USN Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL). The technical manager for the program under which this project was conducted, Fischer Tropsch (F-T) Jet Fuel Toxicity Assessment, was Dr David Mattie. The authors acknowledge the following individuals who also served on a review panel for this program and this project: John Hinz (USAFSAM/OEHTH, Brooks City Base, TX); Gunda Reddy, PhD (USACHPPM, Aberdeen Proving Ground, MD); David Steup, PhD (Shell Oil Company, Houston, TX; Chairman, American Petroleum Institute-Toxicology Task Force); and Errol Zeiger, Ph.D., J.D. (Errol Zeiger Consulting, Chapel Hill, NC).

This study, designated WIL-647001, was conducted in compliance with the United States Environmental Protection Agency (U.S. EPA) Good Laboratory Practice Standards (40 CFR Part 792, 1989); the standard operating procedures of WIL Research Laboratories, LLC; and the protocol as approved by the sponsor. Analytical confirmation of the concentration, homogeneity and stability of the dosing mixture was not supplied by the sponsor and was not conducted as part of this study.

The study protocol was designed to be in general compliance with the U.S. EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.2500 (U.S. EPA, 1998), the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (OECD, 2002).

This animal study was approved by the Air Force Surgeon General's Research Human & Animal Research Panel and the WIL Research Animal Care and Use Committee. 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).

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## 1.0 SUMMARY

The objective of this study was to determine the irritative potential of JP-8, S-8 and a 50/50 volume/volume (v/v) mixture of JP-8 and S-8 following a single exposure to the skin of albino rabbits. Three groups of three New Zealand White (NZW) rabbits received a single, four-hour, occluded exposure and three groups of three NZW rabbits received a single, four-hour, semi-occluded exposure. Doses (0.5 mL) of the test substances were applied to the clipped, unabraded skin. At the completion of all exposures, the bandages were removed and the sites washed. Application sites were evaluated in accordance with the method of Draize (1965) once at approximately 30 to 60 minutes; again at 24, 48 and 72 hours; and at 4, 7 and 14 days after patch removal for sites where irritation persisted.

For single, occluded four-hour exposures of JP-8 to the skin of NZW rabbits, the Primary Dermal Irritation Index (PII) was 2.1 with a descriptive rating classification of moderately irritating. For single, semi-occluded four-hour exposures of JP-8 to the skin of NZW rabbits, the PII was 1.8 with a descriptive rating classification of slightly irritating.

For single, occluded four-hour exposures of S-8 to the skin of NZW rabbits, the PII was 2.3 with a descriptive rating classification of moderately irritating. For single, semi-occluded four-hour exposures of S-8 to the skin of NZW rabbits, the PII was 0.8 with a descriptive rating classification of slightly irritating.

For single, occluded four-hour exposures of JP-8/S-8 mixture to the skin of NZW rabbits, the PII was 1.9 with a descriptive rating classification of slightly irritating. For single, semi-occluded four-hour exposures of JP-8/S-8 (50:50 v/v mixture) to the skin of NZW rabbits, the PII was 1.5 with a descriptive rating classification of slightly irritating.

## 2.0 INTRODUCTION

The U.S. Air Force is in the process of developing alternative fuels in order to decrease foreign oil dependence. Since all new fuels are potentially hazardous to Air Force personnel, they require evaluation. Fischer Tropsch (F-T) fuel is the first alternative jet fuel to be certified for use in the U.S. Air Force fleet. Frequently called S-8 for synthetic jet fuel, this alternative is undergoing toxicological evaluation by the 711<sup>th</sup> Human Performance Wing, Human Effectiveness Directorate, Biosciences and Performance Division, Applied Biotechnology Branch (711 HPW/RHPB). Alternative fuel toxicity will inherently be compared with the toxicity of traditional petroleum distillate JP-8 jet fuel. Dermal irritation studies are an important part of this evaluation.

JP-8 was previously evaluated in a skin irritation protocol designed by Draize (1965). Results from this rabbit skin irritation test ranged from non-irritating to slightly irritating and back to non-irritating (Smith *et al.*, 1981, Kinkead *et al.*, 1992, Wolfe *et al.*, 1996, respectively). 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 antidotal reporting received from operational and medical personnel. Because of the variable results in past jet fuel 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 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 4 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.

This study was designed to re-evaluate the irritative potential of JP-8, as well as the irritation potential of S-8 and a 50/50 volume/volume (v/v) mixture of JP-8 and S-8, following a single exposure to the skin of NZW rabbits. The mixture is of particular importance, as the Air Force intends to use S-8 primarily in a 50/50 blend with traditional JP-8.

### 3.0 MATERIALS AND METHODS

#### 3.1 Test Substances

JP-8, S-8 and a 50/50 volume/volume JP-8/S-8 mixture (Table 1) were provided by Air Force Research Laboratory, Propulsion Directorate, Fuels Branch (AFRL/RZPF). Laboratory test reports for the fuels are presented in Appendix A. The test substances were stored at room temperature in a flame cabinet and were considered stable under these conditions. A reserve sample (approximately 0.5 mL) of each fuel was collected and stored in the Archives of WIL Research Laboratories, LLC. The test substances were used as received.

**Table 1. Test Substances Identification**

|                      | <b>JP-8</b>                | <b>S-8</b>         | <b>JP-8/S-8</b>                        |
|----------------------|----------------------------|--------------------|----------------------------------------|
| <b>Name</b>          | Jet A blend with additives | synthetic jet fuel | JP-8/S-8 mixture (50/50 volume/volume) |
| <b>Batch number</b>  | Jet A W JP 8 ADD           | S8-w additives     | Jet A/S8                               |
| <b>Lot number</b>    | POSF4658                   | POSF5109           | not applicable                         |
| <b>WIL ID number</b> | 08004D                     | 08004E             | 08004F                                 |

#### 3.2 Test Animals

New Zealand White albino rabbits utilized for this study were received in good health from Covance Research Products, Inc. (Kalamazoo, MI). The rabbits were inspected upon receipt, weighed and uniquely identified by a plastic ear tag displaying the animal number. The animals were acclimated to laboratory conditions for a minimum of five days.

Upon arrival, all animals were housed in individual suspended stainless steel cages. The animals were maintained by the animal husbandry staff of WIL Research Laboratories, LLC, in accordance with standard operating procedures.

The certified feed used in this study, PMI Nutrition International, LLC, Certified Rabbit HF LabDiet<sup>®</sup> 5325, was provided at approximately 150 g/day. Municipal water supplying WIL Research Laboratories, LLC was delivered by an automatic watering system, *ad libitum*, throughout the acclimation period and during the study. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study.

The animal room was maintained with controlled temperature, humidity and light (0600 hours to 1800 hours). The room temperature and humidity controls were set to maintain daily averages of  $66 \pm 5^{\circ}\text{F}$  ( $19 \pm 3^{\circ}\text{C}$ ) and  $50 \pm 20\%$  relative humidity. Room temperature and relative humidity were controlled and monitored using the Metasys<sup>®</sup> DDC Electronic Environmental control system (Johnson Controls, Inc., Milwaukee, WI) and were recorded approximately hourly. These data are summarized in Appendix B. Actual mean daily temperature ranged from  $65.5^{\circ}\text{F}$

to 70.7°F (18.6°C to 21.5°C) and mean daily relative humidity ranged from 44.1% to 63.0% during the study.

Animals used in the study were arbitrarily selected from available stock based upon health and body weight. The selected animals were young adults at least 12 weeks old at the initiation of dosing. Body weight values ranged from 1999 g to 2244 g for males and 2099 g to 2274 g for females.

### 3.3 Test Substance Administration

On the day prior to dosing, the hair was removed from the backs and flanks of the rabbits using an electric clipper. A 0.5-mL dose was applied to a dorsal skin area of the trunk of the animals approximately 2.5 cm × 2.5 cm under a 2-ply gauze patch secured in place with Micropore™ tape (Figure 1). For animals in the occluded exposure groups (Groups 1, 3 and 5), the trunk of the animal was wrapped with plastic wrap to occlude the test site. The trunks of animals in both the occluded and semi-occluded groups were then overwrapped with a gauze binder and secured with Dermiform® tape. Plastic restraint collars were applied to the animals to prevent ingestion of the test substance and/or bandages.

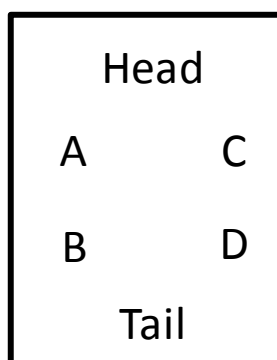
Six groups of three NZW rabbits, one unabraded site per rabbit, were used for this study, per U.S. EPA (1998) and OECD (2002) guidelines. Animals in Groups 1, 3 and 5 received a single, four-hour, occluded exposure. Animals in Groups 2, 4 and 6 received a single, four-hour, semi-occluded exposure (Table 2). Adjacent areas of untreated skin of each animal served as a control for each test site. At the end of four hours, collars and bandages were removed and the sites wiped with disposable paper towels moistened with deionized water.

**Table 2. Study Group Assignments**

| <b>Group Number</b> | <b>Test Substance</b> | <b>Dose Volume (mL)</b> | <b>Exposure Method</b> | <b>Number of Animals</b> |
|---------------------|-----------------------|-------------------------|------------------------|--------------------------|
| <b>1</b>            | JP-8                  | 0.5                     | Occluded               | 3                        |
| <b>2</b>            | JP-8                  | 0.5                     | Semi-occluded          | 3                        |
| <b>3</b>            | S-8                   | 0.5                     | Occluded               | 3                        |
| <b>4</b>            | S-8                   | 0.5                     | Semi-occluded          | 3                        |
| <b>5</b>            | JP-8/S-8*             | 0.5                     | Occluded               | 3                        |
| <b>6</b>            | JP-8/S-8*             | 0.5                     | Semi-occluded          | 3                        |

\*Prepared as a 50/50 (v/v) mixture.

The selected route of administration for this study was direct application to clipped, unabraded skin (dermal). This study was intended to provide information on the health hazards likely to arise from a short-term exposure to JP-8, S-8 or a 50/50 (v/v) mixture of the fuels by the dermal route. The animal model, the NZW albino rabbit, is generally the preferred animal model for acute dermal irritation studies (OECD, 2002). Site of application was randomly selected per rabbit; general location of each site (A through D) is indicated in Figure 1.



**Figure 1. General Locations of Application Sites on the Backs of NZW Rabbits**

### 3.4 Observations

The rabbits were observed twice daily (morning and afternoon) for mortality. The application sites were observed for erythema, edema and other dermal findings once at approximately 30 to 60 minutes and again at 24, 48 and 72 hours after patch removal. If irritation persisted, the sites were read again on study days 4, 7 and 14. Dermal irritation was graded in accordance with the method of Draize (1965; Table 3). The areas of application were clipped free of hair at least 1 hour prior to scoring, as needed during the study, to facilitate accurate dermal observations.

**Table 3. Evaluation of Dermal Reactions**

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

Draize, J.H. (1965)

The Primary Dermal Irritation Index (PII) was calculated from scores recorded once at 30 to 60 minutes and at 24, 48 and 72 hours after patch removal. Days 4, 7 and 14 scores were not part of the calculated PII. 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**

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

Draize, J.H. (1965)

Body weights were obtained and recorded on study day 0 (initiation) and at each rabbit's termination from the study. After study termination, the rabbits were euthanized by intravenous injection of sodium pentobarbital and discarded.

### **3.5 Data Retention**

The sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol (Appendix D). Reserve samples of the test substances, pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC in compliance with regulatory requirements.

## **4.0 RESULTS AND DISCUSSION**

There were no deaths during the study. Dermal findings noted during the study consisted of very slight (grade 1) to slight (grade 2) erythema and edema, and desquamation (Tables 5-10). All dermal findings completely subsided by study termination (study day 14). There were no remarkable body weight changes noted during the study (Table 11).



**Table 5. Individual Dermal Scores for the JP-8 Occluded Test Group**

|                         |      |      | Erythema |          |          |          |          |          |           |
|-------------------------|------|------|----------|----------|----------|----------|----------|----------|-----------|
| Animal                  | Sex  | Site | 0.5-1 h* | 24 h*    | 48 h*    | 72 h*    | 4 d      | 7 d      | 14 d      |
| 55653                   | male | C    | 1        | 2        | 2        | 2        | 2        | 0D       | NA        |
| 55655                   | male | A    | 1        | 2        | 2        | 2        | 2        | 0D       | NA        |
| 55657                   | male | D    | 1        | 2        | 2        | 2        | 2        | 0D       | NA        |
| <b>Individual Total</b> |      |      | <b>3</b> | <b>6</b> | <b>6</b> | <b>6</b> | <b>6</b> | <b>0</b> | <b>NA</b> |
|                         |      |      | Edema    |          |          |          |          |          |           |
| Animal                  | Sex  | Site | 0.5-1 h* | 24 h*    | 48 h*    | 72 h*    | 4 d      | 7 d      | 14 d      |
| 55653                   | male | C    | 1        | 0        | 0        | 0        | 0        | 0        | NA        |
| 55655                   | male | A    | 1        | 0        | 0        | 0        | 0        | 0        | NA        |
| 55657                   | male | D    | 1        | 0        | 0        | 0        | 0        | 0        | NA        |
| <b>Individual Total</b> |      |      | <b>3</b> | <b>0</b> | <b>0</b> | <b>0</b> | <b>0</b> | <b>0</b> | <b>NA</b> |

Notes: h = hours; d = days; \*Test periods used to calculate PII;  $PII = (3+6+6+6)/12 + (3+0+0+0)/12 = 2.1$  = moderately irritating; D = desquamation; NA = not applicable

**Table 6. Individual Dermal Scores for the JP-8 Semi-Occluded Test Group**

|                         |      |      | Erythema |          |          |          |          |          |          |
|-------------------------|------|------|----------|----------|----------|----------|----------|----------|----------|
| Animal                  | Sex  | Site | 0.5-1 h* | 24 h*    | 48 h*    | 72 h*    | 4 d      | 7 d      | 14 d     |
| 55658                   | male | D    | 0        | 1        | 1        | 1        | 1        | 1D       | 0        |
| 55659                   | male | A    | 1        | 2        | 2        | 1        | 1        | 0D       | NA       |
| 55660                   | male | D    | 1        | 1        | 2        | 1        | 1        | 0D       | NA       |
| <b>Individual Total</b> |      |      | <b>2</b> | <b>4</b> | <b>5</b> | <b>3</b> | <b>3</b> | <b>1</b> | <b>0</b> |
|                         |      |      | Edema    |          |          |          |          |          |          |
| Animal                  | Sex  | Site | 0.5-1 h* | 24 h*    | 48 h*    | 72 h*    | 4 d      | 7 d      | 14 d     |
| 55658                   | male | D    | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| 55659                   | male | A    | 1        | 1        | 1        | 1        | 1        | 0        | NA       |
| 55660                   | male | D    | 1        | 0        | 1        | 1        | 1        | 0        | NA       |
| <b>Individual Total</b> |      |      | <b>2</b> | <b>1</b> | <b>2</b> | <b>2</b> | <b>2</b> | <b>0</b> | <b>0</b> |

Notes: h = hours; d = days; \*Test periods used to calculate PII;  $PII = (2+4+5+3)/12 + (2+1+2+2)/12 = 1.8$  = slightly irritating; D = desquamation; NA = not applicable

**Table 7. Individual Dermal Scores for the S-8 Occluded Test Group**

|                         |            |             | <b>Erythema</b> |              |              |              |            |            |             |
|-------------------------|------------|-------------|-----------------|--------------|--------------|--------------|------------|------------|-------------|
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55662                   | male       | A           | 1               | 2            | 2            | 2            | 1          | 0D         | NA          |
| 55664                   | male       | A           | 0               | 1            | 1            | 1            | 1          | 0D         | NA          |
| 55665                   | male       | D           | 1               | 2            | 2            | 2            | 2          | 0D         | NA          |
| <b>Individual Total</b> |            |             | <b>2</b>        | <b>5</b>     | <b>5</b>     | <b>5</b>     | <b>4</b>   | <b>0</b>   | <b>NA</b>   |
|                         |            |             | <b>Edema</b>    |              |              |              |            |            |             |
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55662                   | male       | A           | 2               | 1            | 1            | 1            | 0          | 0          | NA          |
| 55664                   | male       | A           | 1               | 0            | 1            | 1            | 0          | 0          | NA          |
| 55665                   | male       | D           | 1               | 0            | 1            | 1            | 1          | 0          | NA          |
| <b>Individual Total</b> |            |             | <b>4</b>        | <b>1</b>     | <b>3</b>     | <b>3</b>     | <b>1</b>   | <b>0</b>   | <b>NA</b>   |

Notes: h = hours; d = days; \*Test periods used to calculate PII;  $PII = (2+5+5+5)/12 + (4+1+3+3)/12 = 2.3$  = moderately irritating; D = desquamation; NA = not applicable

**Table 8. Individual Dermal Scores for the S-8 Semi-Occluded Test Group**

|                         |            |             | <b>Erythema</b> |              |              |              |            |            |             |
|-------------------------|------------|-------------|-----------------|--------------|--------------|--------------|------------|------------|-------------|
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55666                   | male       | B           | 1               | 0            | 1            | 1            | 1          | 1D         | 0           |
| 55667                   | male       | C           | 0               | 0            | 2            | 1            | 1          | 0D         | NA          |
| 55668                   | male       | D           | 0               | 0            | 0            | 0            | NA         | NA         | NA          |
| <b>Individual Total</b> |            |             | <b>1</b>        | <b>0</b>     | <b>3</b>     | <b>2</b>     | <b>1</b>   | <b>1</b>   | <b>0</b>    |
|                         |            |             | <b>Edema</b>    |              |              |              |            |            |             |
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55666                   | male       | B           | 1               | 0            | 0            | 0            | 0          | 0          | 0           |
| 55667                   | male       | C           | 1               | 0            | 0            | 0            | 0          | 0          | NA          |
| 55668                   | male       | D           | 1               | 0            | 0            | 0            | NA         | NA         | NA          |
| <b>Individual Total</b> |            |             | <b>3</b>        | <b>0</b>     | <b>0</b>     | <b>0</b>     | <b>0</b>   | <b>0</b>   | <b>0</b>    |

Notes: h = hours; d = days; \*Test periods used to calculate PII;  $PII = (1+0+3+2)/12 + (3+0+0+0)/12 = 0.8$  = slightly irritating; D = desquamation; NA = not applicable

**Table 9. Individual Dermal Scores for the JP-8/S-8 Occluded Test Group**

|                         |            |             | <b>Erythema</b> |              |              |              |            |            |             |
|-------------------------|------------|-------------|-----------------|--------------|--------------|--------------|------------|------------|-------------|
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55454                   | male       | A           | 1               | 0            | 1            | 2            | 2          | 2          | 0           |
| 55462                   | male       | D           | 1               | 1            | 1            | 1            | 1          | 0D         | NA          |
| 55463                   | male       | A           | 1               | 1            | 1            | 2            | 2          | 2          | 0           |
| <b>Individual Total</b> |            |             | <b>3</b>        | <b>2</b>     | <b>3</b>     | <b>5</b>     | <b>5</b>   | <b>4</b>   | <b>0</b>    |
|                         |            |             | <b>Edema</b>    |              |              |              |            |            |             |
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55454                   | male       | A           | 1               | 0            | 1            | 2            | 2          | 1          | 0           |
| 55462                   | male       | D           | 1               | 0            | 0            | 0            | 0          | 0          | NA          |
| 55463                   | male       | A           | 1               | 0            | 1            | 2            | 2          | 1          | 0           |
| <b>Individual Total</b> |            |             | <b>3</b>        | <b>0</b>     | <b>2</b>     | <b>4</b>     | <b>4</b>   | <b>2</b>   | <b>0</b>    |

Notes: h = hours; d = days; \*Test periods used to calculate PII;  $PII = (3+2+3+5)/12 + (3+0+2+4)/12 = 1.9$  = slightly irritating; D = desquamation; NA = not applicable

**Table 10. Individual Dermal Scores for the JP-8/S-8 Semi-Occluded Test Group**

|                         |            |             | <b>Erythema</b> |              |              |              |            |            |             |
|-------------------------|------------|-------------|-----------------|--------------|--------------|--------------|------------|------------|-------------|
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55474                   | female     | A           | 0               | 1            | 1            | 1            | 1          | 0D         | NA          |
| 55475                   | female     | D           | 1               | 1            | 1            | 2            | 2          | 0D         | NA          |
| 55482                   | female     | B           | 0               | 1            | 1            | 2            | 1          | 0D         | NA          |
| <b>Individual Total</b> |            |             | <b>1</b>        | <b>3</b>     | <b>3</b>     | <b>5</b>     | <b>4</b>   | <b>0</b>   | <b>NA</b>   |
|                         |            |             | <b>Edema</b>    |              |              |              |            |            |             |
| <b>Animal</b>           | <b>Sex</b> | <b>Site</b> | <b>0.5-1 h*</b> | <b>24 h*</b> | <b>48 h*</b> | <b>72 h*</b> | <b>4 d</b> | <b>7 d</b> | <b>14 d</b> |
| 55474                   | female     | A           | 1               | 0            | 0            | 0            | 0          | 0          | 0           |
| 55475                   | female     | D           | 1               | 0            | 0            | 1            | 1          | 0          | NA          |
| 55482                   | female     | B           | 1               | 0            | 0            | 1            | 1          | 0          | NA          |
| <b>Individual Total</b> |            |             | <b>3</b>        | <b>0</b>     | <b>0</b>     | <b>2</b>     | <b>2</b>   | <b>0</b>   | <b>0</b>    |

Notes: h = hours; d = days; \*Test periods used to calculate PII;  $PII = (1+3+3+5)/12 + (3+0+0+2)/12 = 1.5$  = slightly irritating; D = desquamation; NA = not applicable

**Table 11. Individual Body Weights**

| <b>Fuel</b> | <b>Occlusion</b> | <b>Animal Number</b> | <b>Sex</b> | <b>Day 0 Weight</b> | <b>Day of Termination</b> | <b>Ending Weight</b> |
|-------------|------------------|----------------------|------------|---------------------|---------------------------|----------------------|
| JP-8        | Occluded         | 55653                | M          | 2064                | 7                         | 2313                 |
| JP-8        | Occluded         | 55655                | M          | 2034                | 7                         | 2162                 |
| JP-8        | Occluded         | 55657                | M          | 2144                | 7                         | 2273                 |
|             |                  |                      |            |                     |                           |                      |
| JP-8        | Semi-Occluded    | 55658                | M          | 2016                | 14                        | 2457                 |
| JP-8        | Semi-Occluded    | 55659                | M          | 2120                | 7                         | 2362                 |
| JP-8        | Semi-Occluded    | 55660                | M          | 1999                | 7                         | 2271                 |
|             |                  |                      |            |                     |                           |                      |
| S-8         | Occluded         | 55662                | M          | 2081                | 7                         | 2343                 |
| S-8         | Occluded         | 55664                | M          | 2050                | 7                         | 2291                 |
| S-8         | Occluded         | 55665                | M          | 2049                | 7                         | 2335                 |
|             |                  |                      |            |                     |                           |                      |
| S-8         | Semi-Occluded    | 55666                | M          | 2125                | 14                        | 2411                 |
| S-8         | Semi-Occluded    | 55667                | M          | 2005                | 7                         | 2256                 |
| S-8         | Semi-Occluded    | 55668                | M          | 2210                | 3                         | 2360                 |
|             |                  |                      |            |                     |                           |                      |
| JP-8/S-8    | Occluded         | 55454                | M          | 2244                | 14                        | 2610                 |
| JP-8/S-8    | Occluded         | 55462                | M          | 2132                | 7                         | 2316                 |
| JP-8/S-8    | Occluded         | 55463                | M          | 2228                | 14                        | 2631                 |
|             |                  |                      |            |                     |                           |                      |
| JP-8/S-8    | Semi-Occluded    | 55474                | F          | 2099                | 7                         | 2394                 |
| JP-8/S-8    | Semi-Occluded    | 55475                | F          | 2169                | 7                         | 2572                 |
| JP-8/S-8    | Semi-Occluded    | 55482                | F          | 2274                | 7                         | 2560                 |

Notes: weights in grams, M = male; F = female

Single, occluded four-hour exposures of JP-8 to the skin of NZW rabbits resulted in a Primary Dermal Irritation Index of 2.1 with a descriptive rating classification of moderately irritating. For single, semi-occluded four-hour exposures of JP-8 to the skin of NZW rabbits, the PII was 1.8 with a descriptive rating classification of slightly irritating.

Single, occluded four-hour exposures of S-8 to the skin of NZW rabbits resulted in a PII of 2.3 with a descriptive rating classification of moderately irritating. For single, semi-occluded four-hour exposures of S-8 to the skin of NZW rabbits, the PII was 0.8 with a descriptive rating classification of slightly irritating.

Single, occluded 4-hour exposures of JP-8/S-8 (50:50 v/v mixture) to the skin of NZW rabbits resulted in a PII of 1.9 with a descriptive rating classification of slightly irritating. For single, semi-occluded 4-hour exposures of JP-8/S-8 (50:50 v/v mixture) to the skin of NZW rabbits, the PII was 1.5 with a descriptive rating classification of slightly irritating.

## 5.0 CONCLUSIONS

Based on the Irritation Index, S-8 and the 50:50 blend are either the same or less irritating as JP-8. Therefore, it appears that substituting S-8 for JP-8 or mixing S-8 with JP-8 should not result in a larger dermal irritation hazard than JP-8 alone. As an interesting point, the occluded JP-8/S-8 mixture was less irritating (slight irritation classification) than either JP-8 or S-8 when occluded (moderate irritation classification). However, this doesn't change the recommended handling of the fuels, separately or together; skin protection should be worn as these fuels are at least slightly irritating to the skin under either occluded or semi-occluded conditions.

## 6.0 REFERENCES

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## **APPENDIX A LABORATORY TEST REPORTS**

DEPARTMENT OF THE AIR FORCE  
DET 3, WR-ALC/AFTLA  
2430 C St, Bldg 70 Area B  
Wright-Patterson AFB, OH 45433-7632

LABORATORY TEST REPORT

Submitter's Sample No: POSF4658  
Date Sampled: / /

Lab Report No: F-2004LA04119  
Date Reported: 08/03/2004  
Date Received: 07/07/2004  
Product/Manufacturer/Contractor:

Sample Submitter:  
AFRL/PRSF  
BLDG 490  
1790 LOOP RD N  
WRIGHT-PATTERSON AFB, OH 45433-

Reason for Submission: Specification Conformance Testing  
Product: Aviation Turbine Fuel, Kerosene  
Specification: ASTM D-1655 JET A  
Sample Origin:  
Quantity Represented:

NSN:  
Contract No:  
Batch/Lot:  
Date Manufactured:

| METHOD | TEST                              | LIMITS |        | LAB RESULTS |
|--------|-----------------------------------|--------|--------|-------------|
|        |                                   | MIN    | MAX    |             |
| SPEC\W | Workmanship                       |        | Pass   | Pass        |
| D974   | Total Acid Number, mg KOH/g       |        | 0.10   | 0.00        |
| D1319  | Aromatics, % vol                  |        | 25     | 19          |
| D3227  | Mercaptan Sulfur, % mass          |        | 0.003  | 0.000       |
| D4294  | Total Sulfur, % mass              |        | 0.3    | 0.0         |
| D86    | Distillation                      |        |        |             |
|        | 10% Recovered, deg C              |        | 205    | 180         |
|        | 50% Recovered, deg C              |        | Report | 212         |
|        | 90% Recovered, deg C              |        | Report | 251         |
|        | FBP, deg C                        |        | 300    | 274         |
|        | Residue, % vol                    |        | 1.5    | 1.3         |
|        | Loss, % vol                       |        | 1.5    | 0.9         |
| D56    | Flash Point, deg C                | 38     |        | 51          |
| D1298  | Density @ 15 deg C, kg/m3         | 775    | 840    | 806         |
| D5972  | Freezing Point, deg C (Automatic) |        | -40    | -48         |
| D445   | Viscosity @ -20 deg C, cSt        |        | 8.0    | 5.2         |
| D1322  | Smoke Point, mm                   | 19     |        | 21.0        |
| D1840  | Naphthalenes, % vol               |        | 3      | 2           |
| D130   | Copper Strip Corrosion            |        | 1      | 1a          |
| D3241  | Thermal Stability @ 260 deg C     |        |        |             |
|        | Tube Rating Visual                |        | <3     | 1           |
|        | Change in Press., mm of Hg        |        | 25     | 0           |
| D381   | Existent Gum, mg/100mL            |        | 7.0    | 0.2         |
| D1094  | Water Reaction                    |        | 1B     | 1           |
| D2624  | Conductivity, pS/m                | 200    | 600    | 10##        |
| D5006  | FSII, % vol                       | 0.10   | 0.15   | 0.00##      |
| D3338  | Net Heat of Combustion, MJ/kg     | 42.8   |        | 43.2        |
| 05452  | Particulate Contamination, mg/L   |        | Report | 0.2         |

REMARKS:

Submitter's Sample No: POSF4658  
Lab Report No: F-2004LA04119  
As of : 08/03/2004 08:34:31  
Page 2

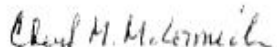
| METHOD | TEST | LIMITS |     | LAB<br>RESULTS |
|--------|------|--------|-----|----------------|
|        |      | MIN    | MAX |                |

---

For information purposes only.  
Particulate contamination = 0.2 mg/L.

Copy To: AFTH

Reported By:

  
CHERYL M. MCCORMICK  
Chemist

Approved By:

  
*For* JEFFREY S. ALLEN  
Chief, Aerospace Fuels Laboratory



DEPARTMENT OF THE AIR FORCE  
HQ AFPT/AFTLA  
2430 C Street, Bldg 70 Area B  
Wright-Patterson AFB, OH 45433-7632

LABORATORY TEST REPORT

Submitter's Sample No: 5109  
Date Sampled: / /

Lab Report No: F-2007LA04036  
Date Reported: 04/25/2007  
Date Received: 04/13/2007  
Product/Manufacturer/Contractor:

Sample Submitter:  
AFRL/PRTG  
BLDG 490  
1790 LOOP ROAD N  
WRIGHT PATTERSON AFB, OH 45433-

Reason for Submission: Fisher-Tropsch Testing  
Product: Aviation Turbine Fuel, Kerosene  
Specification: MIL-T-83133 JP-8  
Sample Origin:  
Quantity Represented:

NSN:  
Contract No:  
Batch/Lot:  
Date Manufactured:

| METHOD   | TEST                                 | LIMITS |        | LAB RESULTS |
|----------|--------------------------------------|--------|--------|-------------|
|          |                                      | MIN    | MAX    |             |
| SPEC\W   | Workmanship                          |        | PASS   | Pass        |
| D3242    | Total Acid Number, mg KOH/g          |        | 0.015  | 0.004       |
| D1319    | Aromatics, % vol                     |        | 25.0   | 0.0         |
| D3227    | Mercaptan Sulfur, % mass             |        | 0.002  | 0.000       |
| D4294-03 | Total Sulfur, % mass                 |        | 0.30   | 0.00        |
| D86      | Distillation                         |        |        |             |
|          | IBP, °C                              |        | REPORT | 150         |
|          | 10% Recovered, °C                    |        | 205    | 173         |
|          | 20% Recovered, °C                    |        | REPORT | 181         |
|          | 50% Recovered, °C                    |        | REPORT | 208         |
|          | 90% Recovered, °C                    |        | REPORT | 246         |
|          | EP, °C                               |        | 300    | 258         |
|          | Residue, % vol                       |        | 1.5    | 1.3         |
|          | Loss, % vol                          |        | 1.5    | 1.2         |
| D93      | Flash Point, °C                      | 38     |        | 50          |
| D5972    | Freezing Point, °C                   |        | -47    | -50         |
| D445     | Viscosity @ -20°C, cSt               |        | 8.0    | 4.4         |
| D3343    | Hydrogen Content, % mass             | 13.4   |        | 15.4        |
| D1322    | Smoke Point, mm                      | 19.0   |        | >40.0       |
| D1840    | Naphthalenes, % vol                  |        | 3.0    | Not Req.    |
| D130     | Copper Strip Corrosion               |        | 1      | 1a          |
| D3241    | Thermal Stability @ 260°C            |        |        |             |
|          | Tube Deposit Rating, Visual          |        | <3     | 1           |
|          | Change in Pressure, mm Hg            |        | 25     | 0           |
| D381     | Existent Gum, mg/100mL               |        | 7.0    | 0.0         |
| D1094    | Water Reaction                       |        | 1B     | 1           |
| D5006    | FSII (DiEGME), % vol                 | 0.10   | 0.15   | 0.06##      |
| D2624    | Conductivity, pS/m                   | 150    | 600    | 53##        |
| D4052    | API Gravity @ 60°F                   | 37.0   | 51.0   | 55.6##      |
| D5001    | Lubricity Test (BOCLE), wear scar mm |        | REPORT | 0.57        |
| D3338    | Heat of Combustion, BTU/lb           | 18400  |        | 18977       |

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BY: *WUP*

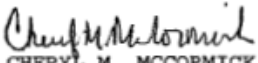
Submitter's Sample No: 5109  
Lab Report No: F-2007LA04036  
As of : 04/25/2007 10:34:02  
Page 2

| METHOD | TEST | LIMITS |     | LAB     |
|--------|------|--------|-----|---------|
|        |      | MIN    | MAX | RESULTS |

---

REMARKS:  
For information purposes only.

Reported By:

  
CHERYL M. MCCORMICK  
Chemist

Approved By:

  
MIGUEL A. ACEVEDO  
Chief, Aerospace Fuels Laboratory

## **APPENDIX B ANIMAL ROOM ENVIRONMENTAL CONDITIONS**

STUDY SPECIFICATIONS: 647001

ROOM SPECIFICATIONS: B ROOM 30  
SPECIES: RABBIT

DATE IN: 06/19/08 TIME IN: 7:00  
DATE OUT: 07/03/08 TIME OUT: 16:00  
LOW TEMPERATURE °F: 61.0 HIGH TEMPERATURE °F: 71.0 LOW HUMIDITY: 30.0  
LOW TEMPERATURE °C: 16.1 HIGH TEMPERATURE °C: 21.7 HIGH HUMIDITY: 70.0

| DATE      | TEMPERATURE |           | HUMIDITY   |
|-----------|-------------|-----------|------------|
|           | MEAN (°F)   | MEAN (°C) | MEAN (%RH) |
| 19-Jun-08 | 65.7        | 18.7      | 49.6       |
| 20-Jun-08 | 65.8        | 18.8      | 48.8       |
| 21-Jun-08 | 65.7        | 18.7      | 51.9       |
| 22-Jun-08 | 65.7        | 18.7      | 50.6       |
| 23-Jun-08 | 65.8        | 18.8      | 50.9       |
| 24-Jun-08 | 65.5        | 18.6      | 52.4       |
| 25-Jun-08 | 65.8        | 18.8      | 55.7       |
| 26-Jun-08 | 66.0        | 18.9      | 63.0       |
| 27-Jun-08 | 65.5        | 18.6      | 60.5       |
| 28-Jun-08 | 65.6        | 18.7      | 61.4       |
| 29-Jun-08 | 65.5        | 18.6      | 56.7       |
| 30-Jun-08 | 65.6        | 18.7      | 53.5       |
| 01-Jul-08 | 65.7        | 18.7      | 48.9       |
| 02-Jul-08 | 65.5        | 18.6      | 50.5       |
| 03-Jul-08 | 65.7        | 18.7      | 56.3       |

| GRAND STATS    | MEAN | MIN  | MAX  |
|----------------|------|------|------|
| TEMPERATURE °F | 65.7 | 65.5 | 66.0 |
| TEMPERATURE °C | 18.7 | 18.6 | 18.9 |
| HUMIDITY (%RH) | 54.0 | 48.8 | 63.0 |
| N DAYS         | 15   |      |      |

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE  
- = VALUE WAS LESS THAN LOW RANGE  
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

STUDY SPECIFICATIONS: 647001

ROOM SPECIFICATIONS: B ROOM 60  
SPECIES: RABBIT

DATE IN: 07/03/08 TIME IN: 16:00  
DATE OUT: 07/10/08 TIME OUT: 16:00  
LOW TEMPERATURE °F: 61.0 HIGH TEMPERATURE °F: 71.0 LOW HUMIDITY: 30.0  
LOW TEMPERATURE °C: 16.1 HIGH TEMPERATURE °C: 21.7 HIGH HUMIDITY: 70.0

| DATE      | TEMPERATURE |           | HUMIDITY   |
|-----------|-------------|-----------|------------|
|           | MEAN (°F)   | MEAN (°C) | MEAN (%RH) |
| 03-Jul-08 | 70.6        | 21.5      | 45.3       |
| 04-Jul-08 | 70.2        | 21.2      | 44.4       |
| 05-Jul-08 | 70.7        | 21.5      | 44.1       |
| 06-Jul-08 | 70.6        | 21.4      | 44.7       |
| 07-Jul-08 | 69.2        | 20.6      | 50.2       |
| 08-Jul-08 | 67.2        | 19.5      | 55.4       |
| 09-Jul-08 | 66.4        | 19.1      | 54.0       |
| 10-Jul-08 | 66.9        | 19.4      | 49.6       |

| GRAND STATS    | MEAN | MIN  | MAX  |
|----------------|------|------|------|
| TEMPERATURE °F | 69.0 | 66.4 | 70.7 |
| TEMPERATURE °C | 20.5 | 19.1 | 21.5 |
| HUMIDITY (%RH) | 48.5 | 44.1 | 55.4 |
| N DAYS         | 8    |      |      |

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE  
- = VALUE WAS LESS THAN LOW RANGE  
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

|                      |           |           |          |             |          |
|----------------------|-----------|-----------|----------|-------------|----------|
| ROOM SPECIFICATIONS: | B ROOM 30 |           |          |             |          |
| SPECIES:             | RABBIT    |           |          |             |          |
| LOW TEMPERATURE:     | 61.0      | DATE IN:  | 06/19/08 |             |          |
| HIGH TEMPERATURE:    | 71.0      | TIME IN:  | 7:00     |             |          |
| LOW HUMIDITY:        | 30.0      | DATE OUT: | 07/03/08 |             |          |
| HIGH HUMIDITY:       | 70.0      | TIME OUT: | 16:00    | TEMPERATURE | HUMIDITY |

ROOM B ROOM 30 SUMMARY

|           |          |      |
|-----------|----------|------|
| MEAN      | 65.7     | 54.1 |
| MIN       | 64.6     | 45.0 |
| MAX       | 68.0     | 72.5 |
| SD        | 0.57     | 5.46 |
| N SAMPLES | 346      | 346  |
| FIRST DAY | 06/19/08 |      |
| LAST DAY  | 07/03/08 |      |
| N DAYS    | 15       |      |

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT  
HUMIDITY UNITS = % RELATIVE HUMIDITY  
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

|                      |           |           |          |             |          |
|----------------------|-----------|-----------|----------|-------------|----------|
| ROOM SPECIFICATIONS: | B ROOM 60 |           |          |             |          |
| SPECIES:             | RABBIT    |           |          |             |          |
| LOW TEMPERATURE:     | 61.0      | DATE IN:  | 07/03/08 |             |          |
| HIGH TEMPERATURE:    | 71.0      | TIME IN:  | 16:00    |             |          |
| LOW HUMIDITY:        | 30.0      | DATE OUT: | 07/10/08 |             |          |
| HIGH HUMIDITY:       | 70.0      | TIME OUT: | 16:00    | TEMPERATURE | HUMIDITY |

ROOM B ROOM 60 SUMMARY

|           |          |      |
|-----------|----------|------|
| MEAN      | 68.9     | 48.7 |
| MIN       | 64.2     | 34.9 |
| MAX       | 73.6     | 64.1 |
| SD        | 2.42     | 6.28 |
| N SAMPLES | 169      | 169  |
| FIRST DAY | 07/03/08 |      |
| LAST DAY  | 07/10/08 |      |
| N DAYS    | 8        |      |

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT  
HUMIDITY UNITS = % RELATIVE HUMIDITY  
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

STUDY 647001 SUMMARY

|           |          |      |
|-----------|----------|------|
| MEAN      | 66.7     | 52.3 |
| MIN       | 64.2     | 34.9 |
| MAX       | 73.6     | 72.5 |
| SD        | 2.09     | 6.26 |
| N SAMPLES | 515      | 515  |
| FIRST DAY | 06/19/08 |      |
| LAST DAY  | 07/10/08 |      |
| N DAYS    | 22       |      |

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT  
HUMIDITY UNITS = % RELATIVE HUMIDITY  
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES



## **APPENDIX C STUDY PROTOCOL**



Study Number: WIL-647001

**PROTOCOL AMENDMENT I**

Sponsor: Naval Health Research Center

A. Title of Study:

Acute Dermal Irritation Study of JP-8 and S-8 in New Zealand White Rabbits

B. Protocol Modification:

1) 7.6 **Test Material Preparation:**

Replace the first line of the second paragraph with the following:

Methods used for preparation of the 50/50 mixture of the test articles will be maintained by the Sponsor.

C. Reason for Protocol Modification:

- 1) The protocol was worded to indicate that the 50/50 mixture of the test articles would be prepared by WIL Research Laboratories, LLC. The mixture was actually provided by the Sponsor.

Approved By:

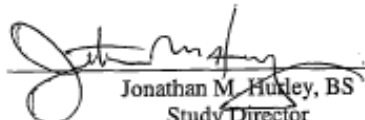
**Naval Health Research Center**

  
LT Dean J Wagner, PhD, MSC, USN  
Sponsor Representative

01 JUL 08  
Date

Prepared By:

**WIL Research Laboratories, LLC**

  
Jonathan M. Huxley, BS  
Study Director

6/25/08  
Date

WIL RESEARCH LABORATORIES, LLC 1407 GEORGE ROAD ASHLAND, OH 44805-9281 (419) 289-8700 FAX (419) 289-3650

**PROTOCOL**

**ACUTE DERMAL IRRITATION STUDY OF  
JP-8 AND S-8 IN NEW ZEALAND WHITE RABBITS**

**Submitted To:**

**Naval Health Research Center**  
Environmental Health Effects Lab  
2729 R St. Bldg. 837 Area B  
Wright Patterson AFB, OH 45433-5707

**WIL Research Laboratories, LLC**  
1407 George Road  
Ashland, OH 44805-9281

**1. OBJECTIVE:**

To determine the irritative potential of the test articles and a mixture of the test articles 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)
- Organization 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 Parts 160 and 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:**

LT Dean J Wagner, PhD, MSC, USN  
Phone: (937) 904-9473  
Fax: (937)-904-9412  
Email: dean.wagner@wpafb.af.mil

**2.2. WIL STUDY DIRECTOR:**

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

**2.3. WIL DEPUTY DIRECTOR:**

Teresa D. Morris, BS  
Senior Operations Manager, Toxicology

**2.4. WIL DEPARTMENTAL RESPONSIBILITIES:**

Christopher P. Chengelis, PhD, DABT  
Director, Toxicology

Eric L. Padgett, PhD  
Assistant Director, Toxicology  
and Head of Juvenile Toxicology

Daniel W. Sved, PhD  
Director, Metabolism and Analytical Chemistry

Theresa M. Rafeld  
Group Manager, Formulations Laboratory

Ronald E. Wilson, BS  
Director, Informational Systems

Sally A. Keets, AS  
Senior Operations Manager, Vivarium

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

Lisa T. Snyder, DVM  
Clinical Veterinarian

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

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

**3. STUDY SCHEDULE:**

Proposed Experimental Start Date:

Proposed Experimental Termination Date:

Proposed Audited Draft Report Date:

**4. TEST ARTICLES:**

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 ARTICLE #1:**

**4.1.1. IDENTIFICATION:**

JP-8 (jet fuel JP-8); Jet A blend with additives

**4.1.2. LOT NUMBER:**

POSF4658

**4.1.3. PURITY:**

Greater than 99%

**4.1.4. STABILITY:**

Stable for years when properly stored.

Expiration date: One year from date of receipt.

**4.1.5. PHYSICAL DESCRIPTION:**

To be documented by WIL Research Laboratories, LLC

**4.1.6. STORAGE CONDITIONS:**

STORE AT ROOM TEMPERATURE. KEEP CONTAINER CLOSED TIGHTLY. USE AND STORE THIS MATERIAL IN COOL, DRY, WELL-VENTILATED AREAS AWAY FROM HEAT, DIRECT SUNLIGHT, HOT METAL SURFACES, AND ALL SOURCES OF IGNITION.

**4.1.7. 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 Sheet for complete available information.

**4.1.8. RETENTION SAMPLES:**

A retention sample of the test article (as received) will be collected in accordance with WIL Research Laboratories, LLC SOP No. T2-001. Dosing preparation samples will not be collected.

**4.1.9. UNUSED TEST ARTICLE:**

The unused portion of the test article will be discarded following the issuance of the final study report.

**4.2. TEST ARTICLE #2:**

**4.2.1. IDENTIFICATION:**

S-8 (Syntroleum S-8) with additives. CAS #: 437986-20-4; Formula: C<sub>7-18</sub>-Alkane Rich Fuel; Molecular Weight: NA, mixture;

**4.2.2. LOT NUMBER:**

POSF5109

**4.2.3. PURITY:**

Greater than 99%

**4.2.4. STABILITY:**

Stable for years when properly stored.

Expiration date: One year from date of receipt.

**4.2.5. PHYSICAL DESCRIPTION:**

To be documented by WIL Research Laboratories, LLC (Colorless liquid)

**4.2.6. STORAGE CONDITIONS:**

STORE AT ROOM TEMPERATURE. KEEP CONTAINER CLOSED TIGHTLY. USE AND STORE THIS MATERIAL IN COOL, DRY, WELL-VENTILATED AREAS AWAY FROM HEAT, DIRECT SUNLIGHT, HOT METAL SURFACES, AND ALL SOURCES OF IGNITION.

**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 Sheet for complete available information.

**4.2.7. RETENTION SAMPLES:**

A retention sample of the test article (as received) will be collected in accordance with WIL Research Laboratories, LLC SOP No. T2-001.

Dosing preparation samples will not be collected.

**4.2.8. UNUSED TEST ARTICLE:**

The unused portion of the test article will be discarded following the issuance of the final study report.

**5. TEST SYSTEM:**

**5.1. SPECIES:**

Albino rabbit

**5.2. BREED:**

New Zealand White

**5.3. SOURCE:**

Covance Research Products, Inc.

(Documentation of the specific breeding facility will be maintained in the study records and included in the final report.)

**5.4. NUMBER ON STUDY:**

Eighteen 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 an average daily temperature of  $66 \pm 5^{\circ}\text{F}$  ( $19 \pm 3^{\circ}\text{C}$ ) and an average daily relative humidity of  $50\% \pm 20\%$ . 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 Rabbit HF LabDiet® 5325 will be offered at approximately 150 g/day during the study. 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 ARTICLE ADMINISTRATION:**

The route of administration will be dermal (clipped, intact skin) in order to evaluate the dermal irritation potential of the test articles. This study is intended to provide information on the health hazards likely to arise from a short-term exposure to the test articles and a 50/50 v/v mixture of the test articles by the dermal route.

#### **7.4. TREATMENT LEVELS:**

The same dose level will be used for all animals (0.5 mL).

#### **7.5. TREATMENT GROUPS:**

Following the acclimation period, animals will be arbitrarily selected from available stock based upon health and body weight and assigned to 6 of 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 Article          | Dose Volume (mL) | Exposure Method | Number of Animals |
|--------------|-----------------------|------------------|-----------------|-------------------|
| 1            | JP-8                  | 0.5              | Occluded        | 3                 |
| 2            | JP-8                  | 0.5              | Unoccluded      | 3                 |
| 3            | S-8                   | 0.5              | Occluded        | 3                 |
| 4            | S-8                   | 0.5              | Unoccluded      | 3                 |
| 5            | JP-8/S-8 <sup>a</sup> | 0.5              | Occluded        | 3                 |
| 6            | JP-8/S-8 <sup>a</sup> | 0.5              | Unoccluded      | 3                 |

<sup>a</sup>Prepared as a 50/50 v/v mixture

#### **7.6. TEST MATERIAL PREPARATION:**

The test articles will be administered undiluted as received and as a 50/50 v/v mixture of the test articles at a dosage of 0.5 mL. The pH will be determined and recorded. (If the pH is 2.0 or less, or 11.5 or greater, the Sponsor will be contacted.) Methods used for preparation of the test article will be documented in the study records and described in the final report. Analysis of dosing preparations will not be performed.

#### **7.7. 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. Animals with dermal abnormalities or injuries will be excluded.

#### **7.8. METHOD OF ADMINISTRATION:**

One site located lateral to the midline of the back will be selected on each rabbit. The test site 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 one of the two test articles or to the mixture of the test articles applied directly to the skin.

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 all animals 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 article and/or wrappings.

After the four hours of exposure, the bandages will be removed and residual test article cleansed from the application site using clean, disposable paper towels moistened with deionized water (as thoroughly as possible without irritating the skin).

## **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 article removal, each test site will be examined and the degree of erythema and edema recorded according to the Draize technique (Appendix). 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 at 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 article and the mixture separately 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 article identification and receipt information, methods, observations, mortality, body weights, individual and summarized dermal scores/findings, classification of the test articles 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 copy of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. One revision will be permitted as part of the cost of the study, from which the Sponsor's reasonable revisions and suggestions will be incorporated into the final report, as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two-month time frame following submission. WIL will submit the final report within one month following receipt of comments. 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, slides, 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:

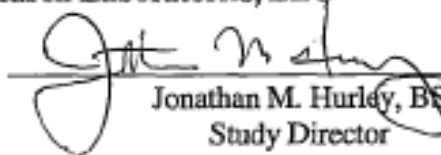
Sponsor approval received by the Study Director via email on June 3, 2008.

### Naval Health Research Center

  
\_\_\_\_\_  
LT Dean J. Wagner, PhD, MSC, USN  
Sponsor Representative

09 JUN 08  
Date

### WIL Research Laboratories, LLC

  
\_\_\_\_\_  
Jonathan M. Hurley, BS  
Study Director

6/4/08  
Date

## APPENDIX

### SCORING CRITERIA FOR DERMAL REACTIONS\*

| <u>Value</u>               | <u>Erythema and Eschar Formation</u>                                                 |
|----------------------------|--------------------------------------------------------------------------------------|
| 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) |
| <hr/>                      |                                                                                      |
| 4                          | Maximum possible erythema score                                                      |
| <br><u>Edema Formation</u> |                                                                                      |
| 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)           |
| <hr/>                      |                                                                                      |
| 4                          | Maximum possible edema score                                                         |
| 8                          | Maximum total possible Primary Irritation Score                                      |

### DESCRIPTIVE RATINGS

#### Mean Primary Dermal Irritation Index

| <u>Range of Values</u> | <u>Descriptive Rating</u> |
|------------------------|---------------------------|
| 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. Association of Food and Drug Officials of the U.S., Topeka, Kansas and the EPA-OPPTS Health Effects Test Guidelines (1998).

## **LIST OF ABBREVIATIONS**

|          |                                                       |
|----------|-------------------------------------------------------|
| EU       | European Union                                        |
| F-T      | Fischer Tropsch                                       |
| NZW      | New Zealand White                                     |
| OECD     | Organisation for Economic Cooperation and Development |
| OPPTS    | Office of Prevention, Pesticides and Toxic Substances |
| PII      | Primary Dermal Irritation Index                       |
| U.S. EPA | U.S. Environmental Protection Agency                  |
| v/v      | volume/volume                                         |