1143-03

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Chemical:	**Gasoline, natural**
PC Code:	**063503**
HED File Code	**13000 Tox Reviews**
Memo Date:	**08/29/96**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

012030

OPP OFFICIAL RECORD AUG 29 1996
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Petroleum Oils. Review of Toxicology Data.
Barcode No: D217192 Submission No.: S474639
Case No.: 819002 Rereg. Case No.: 3004
PC Code No.: 063503 Tox. Chem. No.: 646A

To: Kathryn Davis/Bonnie Adler PM#52
Reregistration Branch
Special Review and Reregistration Division (7508W)

From: Raymond K. Locke, Toxicologist Raymond K. Locke 8/26/96
Section 2, Toxicology Branch I
Health Effects Division (7509C)

Thru: Joycelyn E. Stewart, Ph.D., Section Head for JEStewart 8/27/96
Section 2, Toxicology Branch I
Health Effects Division (7509C)

Registrant: Valent U.S.A. Corporation
Walnut Creek, CA

Action Requested: Review toxicology data (MRID Nos.: 41368806, 41368829, 41368821, 41368822, 41368807, 41368823, and 41368824) submitted to support reregistration of various petroleum oils and indicate whether these data meet the guideline requirements for each study. In addition, determine the need for the Toxicity Endpoint Selection (TES) Committee to examine these data.

Conclusions:

The data presented demonstrate that, under the study conditions, these studies may be classified as follows:

28-Day Dermal Toxicity: In a 28-day dermal toxicity study (MRID 41368822), light neutral oil, Gulf (purity not given) was administered topically to the clipped backs (intact skin) of C3H/HeNCrlBR mice (15/sex/dose) either undiluted or as a 42.5% (w/v) solution in heavy mineral oil. Heavy mineral oil was used as the vehicle control substance. Fifty µL of vehicle, diluted test substance, or undiluted test substance were dermally applied over an approximate area of one square centimeter once daily, three times per week, for four weeks (total of 12 doses). Mortality and moribundity were monitored daily, clinical signs and dermal reactions were checked on dosing days, and body

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weights were taken before study initiation and weekly thereafter. At sacrifice on study day 30, only a gross necropsy was conducted. No analyses of blood, urine, or organ weights were performed; no histopathological examination was conducted on any tissues.

Light neutral oil, Gulf elicited no effects on survival, body weight or body weight gain, or incidence of macroscopic lesions. No adverse clinical signs of systemic or dermal toxicity were noted at any dose tested (up to approximately 2000 mg/kg/day).
The LOEL is greater than the highest dose tested (approximately 2000 mg/kg/day), based on the lack of any toxic effects. The NOEL is equal to or greater than the highest dose tested.

This 28-day dermal toxicity study is classified as unacceptable, but upgradable to acceptable/guideline, with the submission of adequate data on the purity and stability of the test substance. It does not currently satisfy the guideline requirement for a 28-day dermal toxicity study (82-2) in the mouse. Although the study contains several deficiencies, based on the lack of any toxicity at doses up to approximately 2000 mg/kg/day, a repetition of this study would not yield any meaningful additional toxicity data for this substance.

<u>14-Day Dermal Toxicity</u>: In a 14-day dermal toxicity study (MRID 41368829), 100 Paraffine Oil, Gulf (no purity data) was administered topically to the clipped backs (intact skin) of New Zealand White rabbits (3/sex/dose) at dose levels of 0, 1.0 or 2.0 grams/kg body weight/day for 5 days/week for a 2-week period. Three additional high-dose animals/sex were examined following a two-week recovery period, during which no treatment was administered. The vehicle (and control substance) was corn oil; test substance was administered neat for the high-dose (2.0 g/kg/day) group and as a 44.3% (w/v) solution in corn oil for the low-dose (1.0 g/kg/day) group. Dosing volume was maintained at approximately 2.258 ml/kg.

100 Paraffine Oil, Gulf elicited no biologically significant effects on body weight, food consumption, systemic (non-dermal) toxicity, blood hematology or clinical chemistry parameters, organ weights and organ/body weight or organ/brain weight ratios, or gross or microscopic findings at necropsy. Dermal reactions at the treatment site consisted of well defined to severe erythema, with slight to severe edema and eschar formation in the low-dose (1.0 g/kg/day) group; all of these reactions were more severe in the high-dose (2.0 g/kg/day) group and were confirmed with microscopic histopathology. Microscopic examination of skin from the application site of animals in the high-dose group revealed epidermal acanthosis and hyperkeratosis, as well as excessive accumulation of keratin at the skin surface. **The systemic (non-dermal) LOEL is > 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The systemic NOEL is ≥ 2.0 g/kg/day.**

The dermal LOEL is < 1.0 g/kg/day (LDT), based increased incidence of edema and/or erythema and eschar formation at the treatment site with respect to controls (skin lesions were confirmed microscopically at 2.0 g/kg/day). The dermal NOEL is ≤ 1.0 g/kg/day (LDT).

This 14-day dermal toxicity study is classified unacceptable, but ugradable to acceptable/non-guideline, with submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a 21-day dermal toxicity study (82-2) in the rabbit. However, this study provides useful information. Based on the lack of any toxicity other than dermal effects at the treatment site at doses up to approximately 2000 mg/kg/day, a repetition of this study is not required.

<u>14-Day Dermal Toxicity</u>: In a 14-day dermal toxicity study (MRID 41368806), Gulf Orchard Spray 70 (no purity data) was administered topically to the clipped backs (intact skin) of New Zealand White rabbits (3/sex/dose) at dose levels of 0, 1.0 or 2.0 grams/kg body weight/day for 5 days/week for a 2-week period. Three additional high-dose animals/sex were examined following a two-week recovery period, during which no treatment was administered. The vehicle (and control substance) was corn oil; test substance was administered neat for the high-dose (2.0 g/kg/day) group and as a 43.1% (w/v) solution in corn oil for the low-dose (1.0 g/kg/day) group.

Gulf Orchard Spray 70 elicited no biologically significant effects on body weight, food consumption, systemic (non-dermal) toxicity, blood hematology or clinical chemistry parameters, organ weights and organ/body weight or organ/brain weight ratios, or gross or microscopic findings at necropsy. Dermal reactions at the treatment site consisted of slight edema and/or erythema in the low-dose (1.0 g/kg/day) group and much more severe erythema and edema, together with desquamation, in the high-dose (2.0 g/kg/day) group. These dermal observations were confirmed microscopically. Microscopic examination revealed epidermal acanthosis and hyperkeratosis, as well as excessive accumulation of keratin at the skin surface. **The systemic (non-dermal) LOEL is > 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The systemic NOEL is ≥ 2.0 g/kg/day. The dermal LOEL is ≤ 1.0 g/kg/day (LDT), based increased incidence of edema and/or erythema at the treatment site with respect to controls (skin lesions were confirmed microscopically at 2.0 g/kg/day). The dermal NOEL is < 1.0 g/kg/day (LDT).**

This 14-day dermal toxicity study is classified unacceptable, but upgradable to acceptable/non-guideline with the submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a 21-day dermal toxicity study (82-2) in the rabbit. However, this study provides useful information. Based on the lack of any toxicity other than dermal effects at the treatment site at doses up to approximately 2000 mg/kg/day, a repetition of this study is not required.

5-Day Dermal Toxicity: In a 5-day dermal toxicity study (MRID 41368821), Light Neutral Oil, Gulf (no purity data) was administered topically to the clipped backs (intact skin) of Fischer 344 rats (5/sex/dose) at dose levels of 0, 0.85, 1.0 or 2.0 grams/kg body weight/day for 5 days/week for a one-week period (two days of treatment; two days of non-treatment; three days of treatment). The vehicle (and control substance) was heavy paraffin oil; test substance was administered neat for the high-dose (2.0 g/kg/day) and mid-dose (1.0 g/kg/day) groups and as a 42.5% (w/v) solution in heavy paraffin oil for the low-dose (0.85 g/kg/day) group.

Light neutral oil, Gulf elicited no biologically significant effects on survival, clinical or toxicological signs, body weight, food consumption, systemic toxicity, dermal toxicity, or findings at gross necropsy. The **systemic and dermal NOEL ≥ 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The LOEL is > 2.0 g/kg/day.**

This 5-day dermal toxicity study is classified unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance. However, this study provides useful information. Based on the lack of any toxicity at doses up to approximately 2000 mg/kg/day, a repetition of this study is not required.

28-Day Inhalation Toxicity: In a subchronic inhalation toxicity study (MRID 41368824), light neutral oil, Gulf (no purity data) was administered to Fischer 344 rats (10/sex/dose) by whole body inhalation exposure at analytically determined concentrations of 0.0, 0.52, 0.76, or 1.53 g/M^3 (equivalent to 0.0, 0.52, 0.76, or 1.53 mg/L) for six hours per day, five days/week, for a total of 28 days (total of 20 exposures).

No biologically significant effects were noted on survival, body weight, or blood clinical chemistry values. Dose-related clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth, perianal soiling, excessive tearing, and closed eyes. Statistically significant increases were observed in the white blood cell counts in males in all treated groups and in females in the high-dose group. Segmented neutrophils were the white cells most affected by these increases. Absolute lung weights (120-166% control), as well as lung/body (121-183% control) and lung/brain weight ratios (119-167% control), were statistically increased in all animals at all doses tested. Statistically significant increases (110-115% control value) in absolute liver weight were seen at all dose levels in females only, and a statistically significant increase (115% control value) in absolute spleen weight was seen in high-dose females.

Abnormal lung color and/or hemorrhages were noted in the lungs of males in the mid- and high-dose groups and in females in the low-, mid- , and high-dose groups. Accessory spleens and/or abnormally colored spleens were observed in males in the mid- and high-dose

groups and in females in the low- , mid- , and high-dose groups. All treated animals exhibited statistically significant ($p \leq 0.05$) microscopic evidence of peribronchial lymphoid hyperplasia and alveolar macrophage hyperplasia in the lungs. All males and females in the high-dose group exhibited statistically significant incidences of granulomatous pneumonitis of the lungs. Females in the low- and high-dose groups exhibited statistically significant incidences of granulomatous hepatitis of the liver; one high-dose male also exhibited this lesion, but the incidence was not statistically significant. Both high-dose males and females exhibited mononuclear inflammation of the nasal turbinates, but the incidence was statistically significant only for males. Males in the mid- and high-dose groups exhibited granulomatous lympnadenitis of the lymph nodes, but the incidence was statistically significant only at the high-dose; females in the low- , mid- , and high-dose groups exhibited this same lesion, and the incidence was statistically significant for both mid- and high-dose females. **The LOEL is 0.52 g/M^3 (0.52 mg/L; LCT), based on: 1) multiple lung effects (microscopically confirmed peribronchial lymphoid hyperplasia and alveolar macrophage hyperplasia (M+F); increased (120-127% controls; M+F) absolute and relative (both to body and brain weight) lung weight; and abnormal lung color (M); 2) increased white blood cells counts in males (124% control; primarily segmented neutrophils); 3) increased (115% control) absolute liver weight in females; 4) accessory spleens and/or abnormally colored spleens (F); and 5) additional microscopic findings [granulomatous hepatitis (liver) and granulomatous lymphadenitis (lymph nodes)] in females. The NOEL is < 0.52 g/M^3 (LCT).**

This subchronic toxicity study is unacceptable, but upgradable to acceptable/guideline, with the submission of adequate data on the purity and stability of the test substance.

<u>9-Day Inhalation Toxicity</u>: In a subchronic inhalation toxicity study (MRID 41368807), 70 Orchard Spray (no purity data) was administered to Fischer 344 rats (5/sex/dose) by whole body inhalation exposure at analytically determined concentrations of 0.0, 0.70, or 1.60 g/M^3 (equivalent to 0.0, 0.70, or 1.60 mg/L) for six hours per day, for a total of nine exposures.

No biologically significant effects were noted on body weight, hematology parameters, or blood clinical chemistry values. One high-dose (1.60 g/M^3) female died a treatment-related (lung effects) unscheduled death after three exposures. Dose-related clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth, perianal soiling, excessive tearing, and closed eyes. High-dose females also exhibited episodes of labored breathing. 70 Orchard Spray elicited statistically significant increases in absolute weight of the lungs for males (135% control) and females (137% control) in the high-dose (1.6 g/M^3) group, and for females in the low-dose group

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(0.7 g/M^3; 137% control). High-dose (1.6 g/M^3) males exhibited statistically significantly increased (133% control) absolute liver weight, liver/body weight ratio (114% control), liver/brain weight ratio (112% control), lung/body weight ratio (135% control), and lung/brain weight ratio (132% control). High-dose (1.6 g/M^3) females exhibited statistically significantly increased liver/body (116% control) and lung/body (150% control) weight ratios and lung/brain (139% control) weight ratios. Low-dose (0.70 g/M^3) females exhibited increased lung/body (144% control) and lung/brain (141% control) weight ratios. The incidence of microscopically identified hypertrophy and hyperplasia of the alveolar macrophages of the lung was statistically significant ($p \leq 0.05$) for both males and females in the high-dose (1.6 g/M^3) group (5/5 for both sexes). Perivascular/bronchial edema was also observed in both males (2/5) and females (4/5) in the high-dose group, but was statistically significant ($p \leq 0.05$) only for the females. These lesions were especially severe in the one high-dose female that died an unscheduled death. From the data presented, **the LOEL is ≤ 0.70 g/M^3 (LCT; 0.70 mg/mL), based on increased absolute weight of lungs (137% control) and lung/body (144% control) and lung/brain (141% control) weight ratios in females.** Lung lesions were observed microscopically in both males and females in the high-dose (1.60 g/M^3) group, but no microscopic examination was conducted on tissues from low-dose (0.70 g/M^3) animals. **The NOEL < 0.70 g/M^3.**

This subchronic toxicity study is unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a subchronic inhalation study (82-4) in the rat. However, this study is entirely satisfactory for its intended use as a dose range-finding study.

<u>5-Day Inhalation Toxicity</u>: In a subchronic inhalation toxicity study (MRID 41368823), light neutral oil, Gulf (no purity data) was administered to Fischer 344 rats (5/sex/dose) by whole body inhalation exposure at analytically determined concentrations of 0.0, 0.54, 1.70 or 2.79 g/M^3 (equivalent to 0.0, 0.54, 1.70 or 2.79 mg/L) for six hours per day, for a total of five exposures.

No biologically significant effects were noted on body weight. Two females in the high-dose (2.97 g/M^3) group died treatment-related (lung effects) unscheduled deaths; one died after exposure on day 2 and one died on day 4. Treatment-related clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth, perianal soiling, and decreased feces. High-dose females also exhibited episodes of labored breathing, increased respiration, or harsh respiratory sounds. High-dose (2.97 g/M^3) females exhibited abnormally colored contents in the duodenum (2/3), ileum (2/3), and jejunum (2/3). High-dose (2.97 g/M^3) males also exhibited abnormally colored

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contents in the duodenum (2/5), ileum (3/5), and jejunum (2/5). Mid-dose (1.70 g/M^3) males also exhibited abnormally colored contents in the ileum (2/5) and jejunum (2/5). Both high-dose (2.97 g/M^3) males (2/5) and females (1/3) exhibited abnormal liver color, and mid-dose (1.70 g/M^3) females also exhibited abnormal liver color (2/5). Only the lungs from two mid-dose (1.70 g/M^3) females and one high-dose (2.97 g/M^3) male were examined microscopically. The lungs of the high-dose (2.97 g/M^3) male exhibited marked hyperplasia of alveolar macrophages and marked interstitial pneumonitis, as well as slight peribronchial lymphoid hyperplasia and slight perivascular (mononuclear) cuffing. Lungs of the two mid-dose (1.70 g/M^3) females exhibited moderate to severe hyperplasia of alveolar macrophages, severe interstitial pneumonitis, slight to moderate perivascular (mononuclear) cuffing, and none to moderate hemorrhage. From the data presented, **the LOEL is 1.70 g/M^3 (MCT; 1.70 mg/L), based on abnormal colored contents present in the ileum of males and abnormal lung histopathology (moderate to severe hyperplasia of alveolar macrophages, severe interstitial pneumonitis, slight to moderate perivascular (mononuclear) cuffing, and none to moderate hemorrhage) in females. The NOEL is 0.54 g/M^3 (0.54 mg/L; LCT).**

This subchronic toxicity study is unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a subchronic inhalation study (82-4) in the rat. However, this study is entirely satisfactory for its intended use as a dose range-finding study.

Requirement for TES Committee Review: Because both of the petroleum oils tested for inhalation toxicity (light neutral oil, Gulf, and Gulf Orchard Spray 70) elicited adverse lung effects in rats, all of the available dermal and inhalation toxicity data for these substances must be reviewed by the HED's TES Committee whenever HED is requested to prepare the HED chapter of the Reregistration Eligibility Document (RED) for these chemicals.

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Light Neutral Oil, Gulf 28-Day Dermal Toxicity (82-2)

EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke Date 8/12/96
Review Section 2, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. Date 8/26/96
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - Mouse; OPPTS 870.3200, [§82-2]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): Light neutral oil, Gulf (Purity not given)

SYNONYMS: None

CITATION: Zellers, J. and D. Meckley (1984) Four-week repeated dose dermal toxicity range-finding study in mice of light neutral oil. Gulf Life Sciences Center, Pittsburgh, PA. Report Number 1188R, August 2, 1984. MRID 41368822. Unpublished.

SPONSOR: Gulf Refining and Marketing Company Houston, TX

EXECUTIVE SUMMARY:

In a 28-day dermal toxicity study (MRID 41368822), light neutral oil, Gulf (purity not given) was administered topically to the clipped backs (intact skin) of C3H/HeNCrlBR mice (15/sex/dose) either undiluted or as a 42.5% (w/v) solution in heavy mineral oil. Heavy mineral oil was used as the vehicle control substance. Fifty µL of vehicle, diluted test substance, or undiluted test substance were dermally applied over an approximate area of one square centimeter once daily, three times per week, for four weeks (total of 12 doses). Mortality and moribundity were monitored daily, clinical signs and dermal reactions were checked on dosing days, and body weights were taken before study initiation and weekly thereafter. At sacrifice on study day 30, only a gross necropsy was conducted. No analyses of blood, urine, or organ weights were performed; no histopathological examination was conducted on any tissues.

Light neutral oil, Gulf elicited no effects on survival, body weight or body weight gain, or incidence of macroscopic lesions. No adverse clinical signs of systemic or dermal toxicity were noted at any dose tested (up to approximately 2000 mg/kg/day). **The LOEL is greater than the highest dose tested (approximately 2000 mg/kg/day), based on the lack of any toxic effects. The NOEL is equal to or greater than the highest dose tested.**

This 28-day dermal toxicity study is classified as unacceptable, but upgradable to acceptable/guideline, with the submission of adequate data on the purity and stability of the test substance. It does not currently satisfy the guideline requirement for a 28-day dermal toxicity study (82-2) in the mouse. Although the study contains several deficiencies, based on the lack of any toxicity at doses up to approximately 2000 mg/kg/day, a repetition of this study would not yield any meaningful additional toxicity data for this substance.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Light Neutral Oil, Gulf
   Description: Clear, colorless liquid
   Lot/Batch #: Not given
   Purity: Not given
   Stability of compound: Not given
   CAS #: Not given

2. Vehicle and/or positive control: Heavy Paraffin Oil, Laboratory Grade, Saybolt Viscosity 335/365
   CAS No.: 8012-95-1
   Purity: U.S.P. grade
   Source: Fisher Scientific Co., Pittsburgh, PA

3. Test animals: Species: Mouse
   Strain: C3H/HeNCrlBR
   Age and weight at study initiation: 48 days; 16.29-23.24 g.
   Source: Charles River Breeding Laboratories, Inc., Portage, MI
   Housing: Animals were housed individually in stainless steel cages.
   Diet: Purina Certified Rodent Chow (#5002), ad libitum
   Water: Filtered tap water, ad libitum
   Environmental conditions:
   Temperature: 75.03±0.18°F.
   Humidity: 51.50±6.82%
   Air changes: Not reported
   Photoperiod: 12 hours

   Acclimation period: 1 week

B. STUDY DESIGN:

1. In life dates - start: 9/22/83 end: 10/20/83

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN: 28-Day Dermal Toxicity Study of Light Neutral Oil, Gulf in Mice

Test Group	Dose to Animal (μL)[a]	Male	Female
Vehicle Control (Undiluted heavy paraffin oil)	50	15	15
Low (42.5% w/v in heavy paraffin oil)	50	15	15
High (Undiluted light neutral oil)	50	15	15

[a]Dermal doses were maintained at a constant of 50 μL.

Hair was clipped (electric clippers) from the backs of the test animals prior to study initiation and weekly thereafter. Test solutions (50 μL) were applied with a disposable micropipette over an area of approximately one square centimeter of skin. Dosing occurred once daily, three times per week, for four weeks (Monday, Wednesday, and Friday of each week; total of 12 doses over a four-week period).

3. Preparation and analysis of dosing solutions
The 42.5% (w/v) dilution of the light neutral oil, Gulf in vehicle was prepared weekly. Undiluted vehicle and light neutral oil, Gulf were administered neat. No stability nor concentration analyses of the prepared dilutions were performed.

Results - No analyses were conducted.

4. Statistics - Group means and standard deviations were calculated for each sex for each parameter at each time

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period of measurement. Bartlett's test and analysis of variance were then applied. If Bartlett's test indicated that the data were homogeneous, then Dunnett's test was performed. If non-homogeneity was indicated by Bartlett's test, then a modified t-test was performed.

C. METHODS:

1. Observations:

Animals were inspected daily for signs of toxicity and moribundity. Examinations for clinical signs and dermal effects were conducted once daily on days of dosing.

2. Body weight

Animals were weighed prior to study initiation and weekly thereafter.

3. Food consumption

Food consumption was not measured.

4. Ophthalmoscopic examination

No ophthalmological examinations were conducted.

5. Blood was not collected or analyzed.

6. Sacrifice and Pathology

All animals that died (none) and those sacrificed on schedule (Day 30; by carbon dioxide inhalation) were subjected to gross pathological examination. No tissues were collected for histological examination.

II. RESULTS

A. Observations:

1. Toxicity - Light neutral oil, Gulf induced no systemic or dermal toxicity at any dose tested. One high-dose male exhibited a transient subcutaneous mass anterior to the penis for two consecutive dosing days. However, this transient mass (which occurred in one animal only) is not considered to be related to treatment.

2. Mortality - There were no unscheduled deaths; all animals survived to study termination.

B. Body weight and weight gain: There were no statistically

significant treatment-related effects on body weight or body weight gain.

C. Sacrifice and Pathology:

Gross pathology - No treatment-related gross abnormalities were elicited by light neutral oil, Gulf at any dose tested.

III. DISCUSSION

A. Although the specific gravities of light neutral oil, Gulf, its 42.5% (w/v) solution, and heavy paraffin oil (vehicle) are not given, the Merck Index, Tenth Edition, lists the specific gravity for heavy mineral oil as 0.875-0.905 grams/mL. Taking the higher value in the range, the dose of 50 µL is equal to approximately 45.3 mg. Given the average male and female body weights over the treatment period of 0.02294 kg and 0.02026 kg, respectively, the dose of heavy paraffin oil administered represents approximately 1975 mg/kg/day for males and 2235 mg/kg/day for females. Although the light neutral oil, Gulf would be expected to have a specific gravity somewhat less than heavy mineral oil, the resulting calculated dose would still be quite high. Since no toxicity of any kind was observed at these high dosage levels, repetition of this study would add no meaningful additional data on the toxicological properties of the test substance.

B. Study deficiencies - This study contains many deficiencies with respect to Guideline 82-2 requirements: 1) Dosing was accomplished only three times per week for four weeks, rather than the minimum requirement of five times per week for four weeks; 2) No hematology was conducted; 3) No blood clinical chemistry was performed; 4) No organ weights were determined at sacrifice; 5) Purity, stability, and homogeneity data on the test substance and its dilutions were not provided; and 6) No microscopic histopathological examinations were conducted with tissues from animals in the control and high-dose groups.

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EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke Date 8/12/96
Review Section 2, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES, Date 8/26/96
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - Rat; OPPTS 870.3465 [§82-4]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): Light neutral oil, Gulf (Purity not given)

SYNONYMS: None

CITATION: Goode, J. and D. Patrick (1984) Four-week repeated dose inhalation toxicity study in rats of light neutral oil. Chevron Environmental Health Center, Richmond, CA. Report Number 1187, November 16, 1984. MRID 41368824. Unpublished.

SPONSOR: Gulf Refining and Marketing Company Houston, TX

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 41368824), light neutral oil, Gulf (no purity data) was administered to Fischer 344 rats (10/sex/dose) by whole body inhalation exposure at analytically determined concentrations of 0.0, 0.52, 0.76, or 1.53 g/M^3 (equivalent to 0.0, 0.52, 0.76, or 1.53 mg/L) for six hours per day, five days/week, for a total of 28 days (total of 20 exposures).

No biologically significant effects were noted on survival, body weight, or blood clinical chemistry values. Dose-related clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth, perianal soiling, excessive tearing, and closed eyes. Statistically significant increases were observed in the white blood cell counts in males in all treated groups and in females in the high-dose group. Segmented neutrophils were the white cells most affected by these increases. Absolute lung weights (120-166% control), as well as lung/body (121-183% control) and lung/brain weight ratios (119-167% control), were statistically increased in all animals at all doses tested. Statistically significant increases (110-115% control value) in absolute liver weight were seen at all dose levels in females only, and a statistically significant increase (115% control value) in absolute spleen weight was seen in high-dose females.

Abnormal lung color and/or hemorrhages were noted in the lungs of males in the mid- and high-dose groups and in females in the low-, mid- , and high-dose groups. Accessory spleens and/or abnormally colored spleens were observed in males in the mid- and high-dose groups and in females in the low- , mid- , and high-dose groups. All treated animals exhibited statistically significant ($p \leq 0.05$) microscopic evidence of peribronchial lymphoid hyperplasia and alveolar macrophage hyperplasia in the lungs. All males and females in the high-dose group exhibited statistically significant incidences of granulomatous pneumonitis of the lungs. Females in the low- and high-dose groups exhibited statistically significant incidences of granulomatous hepatitis of the liver; one high-dose male also exhibited this lesion, but the incidence was not statistically significant. Both high-dose males and females exhibited mononuclear inflammation of the nasal turbinates, but the incidence was statistically significant only for males. Males in the mid- and high-dose groups exhibited granulomatous lympnadenitis of the lymph nodes, but the incidence was statistically significant only at the high-dose; females in the low- , mid- , and high-dose groups exhibited this same lesion, and the incidence was statistically significant for both mid- and high-dose females. **The LOEL is 0.52 g/M^3 (0.52 mg/L; LCT), based on: 1) multiple lung effects (microscopically confirmed peribronchial lymphoid hyperplasia and alveolar macrophage hyperplasia (M+F); increased (120-127% controls; M+F) absolute and relative (both to body and brain weight) lung weight; and abnormal lung color (M); 2) increased white blood cells counts in males (124% control; primarily segmented neutrophils); 3) increased (115% control) absolute liver weight in females; 4) accessory spleens and/or abnormally colored spleens (F); and 5) additional microscopic findings [granulomatous hepatitis (liver) and granulomatous lymphadenitis (lymph nodes)] in females. The NOEL is < 0.52 g/M^3 (LCT).**

This subchronic toxicity study is unacceptable, but upgradable to acceptable/guideline, with the submission of adequate data on the purity and stability of the test substance.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Light Neutral Oil, Gulf
   Description: Clear, colorless oil
   Lot/Batch #: Not given
   Purity: Not given
   Stability of compound: Not given
   CAS #: Not given

2. Vehicle and/or positive control: None used
Lot/Batch # None

3. Test animals: Species: Rat
Strain: Fischer 344
Age and weight at study initiation: 12 weeks; 137-279 g
Source: Charles River Breeding Laboratories, Inc.
Portage, MI
Housing: Individual in stainless steel cages
Diet: Purina Certified Rodent Diet #5002, ad libitum
(except during exposure)
Water: Tap water, ad libitum
Environmental conditions: Temperature: 73.1±1.1°F
Humidity: 52±5%
Air changes: Not reported
Photoperiod: 12 hours dark/light
Acclimation period: One week

B. STUDY DESIGN:

1. In life dates - start: 10/19/83 end: 11/16/83

2. Animal assignment

Animals were assigned randomly by computer program to the test groups in Table 1.

TABLE 1: STUDY DESIGN[a]

Test group	Nominal Conc. (g/M^3)	Analytical Conc. (g/M^3)[b]	MMAD μM	GSD μM	Rats/sex
Control	0	0.04±0.02[c,d]	--[e]	--[e]	10
Low (LCT)	0.50	0.52±0.04	4.3±0.6	2.1±0.3	10
Mid (MCT)	0.75	0.76±0.11	5.0±0.3	2.0±0.3	10
High (HCT)	1.50	1.53±0.16	5.3±0.4	1.9±0.2	10

[a]Data extracted from pages 4, 8 and 12 of this submission (MRID 41368824).
[b]Time-weighted average
[c]Control analytical background
[d]Mean and standard deviation from the mean
[e]Insufficient number of particles for determination

2. **Generation of the test atmosphere and description of the chamber:** The test substance was aerosolized in a stainless steel glove box fitted to the inlet port of the exposure chamber. The glove box was fitted with a spraying system atomizer with a #1650 liquid nozzle and a

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#64 air nozzle. Dry, filtered compressed air was used to transport the aerosol to the exposure chamber. Large diameter particles were trapped by impaction on glass plates inserted into glass beakers which were placed between the aerosolizer and the exposure chamber; the large particles were thus collected for reaerosolization. Stainless steel tubing was used for all transport applications. Glass and stainless steel ($6M^3$) whole-body exposure chambers were used, and all animals for a given treatment group were placed in a single chamber, and exposures were conducted for 6 hours/day for 5 days/week for 28 days. Exposure chambers were changed weekly with respect to treatment groups. Time to equilibrium (t90--theoretical time to reach 90% of the target concentration) was 11-15 minutes.

Test atmosphere concentration: Chamber concentrations were determined for each exposure gravimetrically using 47-mm cellulose acetate filters. Pretest studies demonstrated that the vapor phase concentration of the test substance was below the limits of detection. The methodology used in the pretest studies consisted of collecting the vapor phase of the test substance behind the filter holder of a fritted glass impinger containing 90 mL of cyclohexane as the collecting solvent. High-pressure liquid chromatographic analyses, using known standards, were then used to detect any test substance in the vapor phase. Chamber concentrations (g/M^3) of the test substance were calculated by dividing the weight of test substance collected on the filters by the sample volume. Results are presented in Table 1 above.

Particle size determination: Daily particle size determinations were conducted for each exposure chamber, using an aerodynamic particle sizer (TSI, Inc., Model APS 33). Data were reported as mass median aerodynamic diameter (MMAD) with the geometric standard deviation (diameter at 84% divided by diameter at 50%). Results are presented in Table 1 above.

3. Statistics - The means and standard deviations from the mean were calculated for all parameters investigated. Differences between group mean body weights were analyzed with Dunnett's test. For clinical chemistry and hematology values, Barlett's test and analysis of variance were performed; if homogeneity was indicated, Dunnett's test was performed; if nonhomogeneity was indicated, a modified t-test was conducted. Organ weight data were analyzed in the same manner as clinical chemistry and hematology values, except that an ANOVA test was additionally conducted. Microscopic findings were analyzed by a two-tailed Kolmogorov-Smirnov analysis.

C. METHODS:

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1. Observations:

Animals were inspected twice daily on weekdays and once daily on weekends for signs of toxicity and mortality.

2. Body weight

Animals were weighed prior to the first exposure and weekly thereafter.

3. Food consumption

Food consumption was not measured.

4. Ophthalmoscopic examination

Eyes were not examined.

5. Blood was collected from non-fasted, anesthetized animals via the orbital sinuses for hematology and clinical analysis (from all animals prior to study initiation and from all animals at terminal sacrifice). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count[a]
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines
[a]Determined only if signs of anemia were observed.

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b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
			Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was not analyzed.

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals survived to study termination, were sacrificed on schedule, and were subjected to gross pathological examination; the CHECKED (X) tissues were collected for histological examination. Only the underlined organs were examined for the high- (1.53 g/M^3) and low-dose (0.52 g/M^3) groups only. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*		Bone marrow*		
X	Stomach*	X	Lymph nodes* (mesenteric)	X	Spinal cord (3 levels)[T] (lumbar)
X	Duodenum*				
X	Jejunum*	XX	Spleen*	X	Pituitary*
X	Ileum*	X	Thymus*	X	Eyes (optic n.)[T]
	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
	Rectum*	XX	Kidneys*+	XX	Adrenal gland*
XX	Liver*+	X	Urinary bladder*		Lacrimal gland[T]
	Gall bladder*	XX	Testes*+	X	Mammary gland[T]
X	Pancreas*	X	Epididymides	X	Parathyroids*++
		X	Prostate	X	Thyroids*++
	RESPIRATORY	X	Seminal vesicle		
X	Trachea*	X	Ovaries		OTHER
XX	Lung*	X	Uterus*	X	Bone
X	Nose (turbinates)	X	Vagina	X	Skeletal muscle
	Pharynx			X	Skin
	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines.
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ.
++ Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. Toxicity - The most frequently noted clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth. These signs were dose-related. At the highest dose tested (1.53 g/M^3), more frequent red nasal discharges, perianal soiling, excessive tearing, and closed eyes were also noted.

2. Mortality - All animals survived until scheduled sacrifice.

B. Body weight and weight gain Statistically significant decreases in mean body weight were seen in both males and females at several dose levels. However, mean body weights never were less than 85% of the appropriate control values. Therefore, the biological significance of these small decreases is unknown. Representative body weight data are presented in the following Table 2:

Table 2. Body Weights of Animals Exposed to Light Neutral Oil, Gulf for 28 Days by Inhalation[a,b]

Study Day	Sex	0 (Control)	0.52 g/M^3	0.76 g/M^3	1.53 g/M^3
1	Male	256.79± 13.83[c] (100)[d]	253.63± 10.63 (99)	243.90± 14.72 (95)	245.99± 11.07 (96)
	Female	154.07± 0.04 (100)	156.28± 6.66 (101)	152.08± 6.81 (99)	144.34± 9.06** (94)
8	Male	274.32± 12.06 (100)	266.22± 9.14 (97)	232.60± 14.96** (85)	230.71± 12.19** (84)
	Female	164.30± 11.73 (100)	163.17± 7.22 (99)	159.66± 7.22 (97)	151.30± 9.34** (92)
15	Male	283.19± 12.69 (100)	273.80± 11.09 (97)	264.48± 11.94** (93)	264.30± 11.37** (93)
	Female	167.66± 7.00 (100)	164.72± 7.26 (98)	148.26± 9.10 (88)	158.00± 9.22** (89)
22	Male	299.30± 12.99 (100)	303.28± 12.56 (101)	278.60± 11.62** (93)	272.82± 11.13** (91)
	Female	173.21± 7.60 (100)	172.01± 7.30 (99)	168.31± 9.99 (97)	159.60± 4.60** (92)
28	Male	316.40± 13.50 (100)	306.20± 10.34 (97)	293.80± 12.17** (93)	286.07± 10.04** (90)
	Female	180.66± 0.37 (100)	179.90± 8.90 (100)	172.31± 11.07 (95)	166.94± 3.73** (92)

[a]Data taken from pages 21 and 22 of this submission (MRID 41368824).
[b]Submitted data were almost illegible; therefore the figures presented in Table 2 represent the reviewing toxicologist's best estimate.
[c]Mean and standard deviation from the mean.
[d]Figures in parentheses represent percentages of the appropriate control value.
**Indicates that $p \leq 0.01$.

C. Food consumption - Food consumption was not monitored.

D. Ophthalmoscopic examination - Eye examinations were not conducted.

E. Blood work

1. Hematology - The only treatment-related effects observed in hematology values were statistically significant increases in the white blood cell counts in males (124-127% control value) in all treated groups and in females (121% control value) in the high-dose (1.53 g/M^3) group. Differential white blood cell counts demonstrated that segmented neutrophils were the white cells most affected by these increases. Segmented neutrophils were statistically significantly increased in males (235% of control value) at the high-dose level (1.53 g/M^3) and in females at both the mid- (0.76 g/M^3; 193% control value) and high-dose (1.53 g/M^3; 293% control value) levels. These data are presented in the following Table 3.

Table 3. Hematological Values for Animals Exposed for 28 Days to Light Neutral Oil, Gulf by Inhalation[a]

Parameter Measured	Sex	0 (Control)	0.52 g/M^3	0.76 g/M^3	1.53 g/M^3
White Blood Cell Count (10^3 per cubic mM)	Male	6.46± 1.40[b] (100)[c]	7.99± 0.64** (124)	8.05± 1.03** (125)	8.21± 1.08** (127)
	Female	5.82± 1.07 (100)	6.08± 1.02 (104)	5.96± 0.30 (102)	7.03± 0.78* (121)
Segmented Neutrophils (10^3 per cubic mM)	Male	0.877± 0.292 (100)	1.469± 0.609 (168)	1.428± 0.514 (163)	2.059± 0.775** (235)
	Female	0.591± 0.121 (100)	0.905± 0.341 (153)	1.141± 0.277** (193)	1.733± 0.492** (293)

[a]Data taken from pages 39 and 54 of this submission (MRID 41368824).
[b]Means and standard deviations from the means.
[c]Numbers in parentheses represent percentages of the appropriate control values.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

2. Clinical chemistry - With the exception of three sporadic and dose-unrelated statistically significant values [decreased (92% control) albumin in high-dose females; decreased (88% control) blood urea nitrogen in high-dose males; and decreased (85% control) albumin/globulin ratio in high-dose females], the clinical chemistry values in all treated animals were comparable to those of appropriate controls.

F. Urinalysis - Urinalyses were not conducted.

G. Sacrifice and pathology

1. Organ weight - Treatment-related increases in absolute weight of the lungs were observed in both males and females at all dose levels of light neutral oil, Gulf tested. These increases (120-166% control values) were statistically significant at all dose levels and were dose-related. Statistically significant increases (110-115% control value) in absolute liver weight were seen at all dose levels in females only. In addition, a statistically significant increase (115% control value) in absolute spleen weight was seen in high-dose (1.53 g/M^3) females, but not in high-dose males. These data are presented in the following Table 4.

Table 4. Absolute Organ Weights of Organs from Animals Exposed to Light Neutral Oil, Gulf for 28 Days by Inhalation[a]

Organ	Sex	0 (Control)	0.52 g/M^3	0.76 g/M^3	1.53 g/M^3
Lungs	Male	1.1427± 0.0654[b] (100)[c]	1.4565± 0.1723** (127)	1.5181± 0.0577** (133)	1.8929± 0.0722** (166)
	Female	0.8856± 0.0602 (100)	1.0540± 0.0671** (120)	1.1394± 0.0711** (129)	1.3849± 0.0733** (156)
Spleen	Male	0.5938± 0.0289 (100)	0.5909± 0.0385 (100)	0.5721± 0.0237 (96)	0.5757± 0.0367 (97)
	Female	0.4006± 0.0196 (100)	0.4220± 0.0341 (105)	0.4121± 0.0584 (103)	0.4622± 0.0211** (115)
Liver	Male	11.9297± 0.5689 (100)	12.4176± 1.2411 (104)	11.4150± 0.7322 (96)	11.4115± 1.0611 (96)
	Female	6.2656± 0.3578 (100)	7.2053± 0.4133** (115)	6.8997± 0.7993* (110)	7.1029± 0.6033** (113)

[a]Data taken from pages 57 and 58 of this submission (MRID 41368824).
[b]Means and standard deviations from the means.
[c]Numbers in parentheses represent percentages of the appropriate control values.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

Organ/body weight ratios demonstrated increased weight of the lungs in all treated males and females (121-183% of control ratios). Increased spleen/body weight ratios were seen in both

high-dose (1.53 g/M^3) males and females (107-125% control ratios). Increased liver/body weight ratios were observed in low-dose (0.52 g/M^3) males (107% control ratio) and at all dose levels in females (115-123% control ratio). Brain/body weight ratios were increased in mid- (0.76 g/M^3; 106% control ratio) and high-dose (1.53 g/M^3; 110% control ratio) males and in high-dose (1.53 g/M^3; 113% control ratio) females. Increased adrenals/body weight ratios were observed in high-dose (1.53 g/M^3; 126% control ratio) males and mid- (0.76 g/M^3; 123% control ratio) and high-dose (1.53 g/M^3; 120% control ratio) females. Representative organ weight/body weight ratio data are presented in the following Table 5.

Table 5. Organ/Body Weight Ratios for Animals Exposed to Light Neutral Oil, Gulf for 28 Days by Inhalation[a]

Organ	Sex	0 (Control)	0.52 g/M^3	0.76 g/M^3	1.53 g/M^3
Lung	Male	0.3613± 0.0180[b] (100)[c]	0.4752± 0.0485** (132)	0.5148± 0.0178** (142)	0.6605± 0.0304** (183)
	Female	0.4910± 0.0395 (100)	0.5963± 0.0362** (121)	0.6622± 0.0340** (135)	0.8304± 0.0517** (169)
Spleen	Male	0.1877± 0.0063 (100)	0.1929± 0.0098 (103)	0.1949± 0.0093 (104)	0.2010± 0.0166* (107)
	Female	0.2221± 0.0179 (100)	0.2387± 0.0179 (107)	0.2385± 0.0248 (107)	0.2773± 0.0193** (125)
Liver	Male	3.7719± 0.1598 (100)	4.0509± 0.3177* (107)	3.8850± 0.1897 (103)	3.9739± 0.2782 (105)
	Female	3.4687± 0.1342 (100)	4.0738± 0.1365** (117)	3.9945± 0.2275** (115)	4.2515± 0.2760** (123)
Brain	Male	0.5770± 0.0185 (100)	0.5948± 0.0110 (103)	0.6114± 0.0275** (106)	0.6329± 0.0178** (110)
	Female	0.9460± 0.0493 (100)	0.9650± 0.0376 (102)	0.9877± 0.0706 (104))	1.0137± 0.0328* (113)
Adrenals	Male	0.0123± 0.0017 (100)	0.0118± 0.0016 (96)	0.0128± 0.0022 (104)	0.0155± 0.0015** (126)

Adrenals	Female	0.0235± 0.0056 (100)	0.0252± 0.0027 (107)	0.0290± 0.0037* (123)	0.0282± 0.0022* (120)

[a]Data taken from pages 59 and 60 of this submission (MRID 41368824).
[b]Means and standard deviations from the means.
[c]Numbers in parentheses represent percentages of the appropriate control values.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

Organ/brain weight ratios demonstrated increased (119-167% control ratios) weight of the lungs in both males and females at all dose levels tested. Increased liver/brain weight ratios (112-116% control ratio) were observed for females at all treatment levels, but not in males. Increased adrenals/brain weight ratios were seen for mid-dose (0.72 g/M^3; 120% control ratio) females and high-dose (1.53 g/M^3; 115% control ratio) males. Representative organ/brain weight ratio data are presented in the following Table 6.

Table 6. Organ/Brain Weight Ratios for Animals Exposed to Light Neutral Oil, Gulf for 28 Days by Inhalation[a]

Organ	Sex	0 (Control)	0.52 g/M^3	0.76 g/M^3	1.53 g/M^3
Lungs	Male	0.6266± 0.0348[b] (100)[c]	0.7997± 0.0889** (128)	0.8477± 0.0399** (135)	1.0436± 0.0391** (167)
	Female	0.5192± 0.0339 (100)	0.6189± 0.0450** (119)	0.6725± 0.0438** (130)	0.8193± 0.0457** (158)
Liver	Male	6.5407± 0.2813 (100)	6.8171± 0.6104 (104)	6.3676± 0.4476 (97)	6.2873± 0.5307 (96)
	Female	3.6509± 0.2074 (100)	4.2267± 0.1999** (116)	4.0725± 0.4835* (112)	4.1981± 0.3123** (115)
Adrenals	Male	0.0213± 0.0029 (100)	0.0198± 0.0026 (93)	0.0209± 0.0035 (98)	0.0245± 0.0019* (115)
	Female	0.0243± 0.0053 (100)	0.0262± 0.0032 (108)	0.0292± 0.0036* (120)	0.0280± 0.0025 (115)

[a]Data taken from pages 61 and 62 of this submission (MRID 41368824)
[b]Means and standard deviations from the means.
[c]Numbers in parentheses represent percentages of the appropriate control

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values.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

2. Gross pathology - Light neutral oil, Gulf caused abnormal lung color and/or hemorrhages in the lungs of males in the mid- (0.76 g/M^3; 3/10) and high-dose (1.53 g/M^3; 9/10) groups and in females in the low- (0.52 g/M^3; 2/10), mid- (0.76 g/M^3; 3/10), and high-dose (1.53 g/M^3; 10/10) groups. Accessory spleens and/or abnormally colored spleens were observed in males in the mid- (0.76 g/M^3; 1/10) and high-dose (1.53 g/M^3; 1/10) groups and in females in the low- (0.52 g/M^3; 1/10), mid- (0.76 g/M^3; 1/10), and high-dose (1.53 g/M^3; 2/10) groups. Although liver nodules were observed in females in the low- (0.52 g/M^3; 2/10) and mid-dose (0.76 g/M^3; 2/10) groups, they were not observed in the high-dose (1.53 g/M^3) group, and are therefore not considered to be treatment-related.

3. Microscopic pathology

a) Non-neoplastic - All animals (10/10 of each group) treated with light neutral oil, Gulf exhibited statistically significant ($p \leq 0.05$) microscopic evidence of peribronchial lymphoid hyperplasia and alveolar macrophage hyperplasia in the lungs. All males and females (10/10 of each group) in the high-dose (1.53 g/M^3) group exhibited statistically significant incidences of granulomatous pneumonitis of the lungs. Females in the low- (0.52 g/M^3; 2/10) and high-dose (1.53 g/M^3; 7/10) groups exhibited statistically significant incidences of granulomatous hepatitis of the liver; one high-dose male also exhibited this lesion, but the incidence was not statistically significant. Both high-dose males (9/10) and females (4/10) exhibited mononuclear inflammation of the nasal turbinates, but the incidence was statistically significant only for males. Males in the mid- (0.76 g/M^3; 1/10) and high-dose (1.53 g/M^3; 7/10) groups exhibited granulomatous lympnadenitis of the lymph nodes, but the incidence was statistically significant only the high-dose males; females in the low- (0.52 g/M^3; 1/10), mid- (0.76 g/M^3; 6/10), and high-dose (1.53 g/M^3; 8/10) exhibited this same lesion, and the incidence was statistically significant for both mid- and high-dose females. Treatment-unrelated lesions included granulomatous inflammation of the adrenals in two high-dose (1.53 g/M^3) males and testicular atrophy and aspermatogenesis in one high-dose male.

b) Neoplastic - No neoplastic lesions were observed in the study.

III. **DISCUSSION**

A. This study, which has several minor deficiencies, nevertheless demonstrates serious toxicity elicited by light neutral oil, Gulf when exposure is by inhalation. From the data presented, **the LOEL is 0.52 g/M^3 (0.52 mg/L; LCT), based on: 1) multiple lung effects (microscopically confirmed peribronchial lymphoid hyperplasia and alveolar macrophage hyperplasia (M+F); increased (120-127% controls; M+F) absolute and relative (both to body and brain weight) lung weight; and abnormal lung color (M); 2) increased white blood cells counts in males (124% control; primarily segmented neutrophils); 3) increased (115% control) absolute liver weight in females; 4) accessory spleens and/or abnormally colored spleens (F); and 5) additional microscopic findings [granulomatous hepatitis (liver) and granulomatous lymphadenitis (lymph nodes)] in females. The NOEL is < 0.52 g/M^3 (LCT).**

B. Study deficiencies This study has the following minor deficiencies: food consumption was not measured; no measure of blood-clotting potential was undertaken; no ophthalmological examinations were conducted; no measurements for calcium, chloride, phosphorus, total bilirubin, or fasted blood glucose were included in the hematology aspect of the study; and the following organs were not examined: cecum, rectum, and bone marrow.

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EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke, Date 8/22/94
Review Section 2, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES, Date 8/24/94
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - Rat; OPPTS 870.3465 [§82-4]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): Light Neutral Oil, Gulf (No purity data)

SYNONYMS: None

CITATION: Goode, J. and D. Patrick (1984) Five-day repeated dose inhalation toxicity study in rats of light neutral oil. Gulf Life Sciences Center, Pittsburgh, PA. Report Number 1185, July 2, 1984. MRID 41368823. Unpublished.

SPONSOR: Gulf Oil Refining and Marketing Company Houston, TX

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 41368823), light neutral oil, Gulf (no purity data) was administered to Fischer 344 rats (5/sex/dose) by whole body inhalation exposure at analytically determined concentrations of 0.0, 0.54, 1.70 or 2.79 g/M^3 (equivalent to 0.0, 0.54, 1.70 or 2.79 mg/L) for six hours per day, for a total of five exposures.

No biologically significant effects were noted on body weight. Two females in the high-dose (2.97 g/M^3) group died treatment-related (lung effects) unscheduled deaths; one died after exposure on day 2 and one died on day 4. Treatment-related clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth, perianal soiling, and decreased feces. High-dose females also exhibited episodes of labored breathing, increased respiration, or harsh respiratory sounds. High-dose (2.97 g/M^3) females exhibited abnormally colored contents in the duodenum (2/3), ileum (2/3), and jejunum (2/3). High-dose (2.97 g/M^3) males also exhibited abnormally colored contents in the duodenum (2/5), ileum (3/5), and jejunum (2/5). Mid-dose (1.70 g/M^3) males also exhibited abnormally colored contents in the ileum (2/5) and jejunum (2/5). Both high-dose (2.97 g/M^3) males (2/5) and females (1/3) exhibited abnormal liver color, and mid-dose (1.70 g/M^3) females also exhibited abnormal liver color (2/5). Only the lungs from two mid-dose

(1.70 g/M^3) females and one high-dose (2.97 g/M^3) male were examined microscopically. The lungs of the high-dose (2.97 g/M^3) male exhibited marked hyperplasia of alveolar macrophages and marked interstitial pneumonitis, as well as slight peribronchial lymphoid hyperplasia and slight perivascular (mononuclear) cuffing. Lungs of the two mid-dose (1.70 g/M^3) females exhibited moderate to severe hyperplasia of alveolar macrophages, severe interstitial pneumonitis, slight to moderate perivascular (mononuclear) cuffing, and none to moderate hemorrhage. From the data presented, **the LOEL is 1.70 g/M^3 (MCT; 1.70 mg/L), based on abnormal colored contents present in the ileum of males and abnormal lung histopathology (moderate to severe hyperplasia of alveolar macrophages, severe interstitial pneumonitis, slight to moderate perivascular (mononuclear) cuffing, and none to moderate hemorrhage) in females. The NOEL is 0.54 g/M^3 (0.54 mg/L; LCT).**

This subchronic toxicity study is unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a subchronic inhalation study (82-4) in the rat. However, this study is entirely satisfactory for its intended use as a dose range-finding study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Light Neutral Oil, Gulf
   Description: Light yellow liquid
   Lot/Batch #: Not given
   Purity: Not given
   Stability of compound: Not given
   CAS #: Not given

2. Vehicle and/or positive control: None used
   Lot/Batch # None

3. Test animals: Species: Rat
   Strain: Fischer 344
   Age and weight at study initiation: 15 weeks; 153-301 g
   Source: Charles River Breeding Laboratories, Inc.
   Portage, MI
   Housing: Individually in stainless steel cages
   Diet: Purina Certified Rodent Diet #5002, *ad libitum* (except during exposure)
   Water: Tap water, *ad libitum*
   Environmental conditions: Temperature: 73.2±0.3°F
   Humidity: 47±13%
   Air changes: Not reported
   Photoperiod: 12 hours dark/light

Acclimation period: Time period not given

B. STUDY DESIGN:

1. In life dates - start: 9/19/83 end: 9/23/83

2. Animal assignment

Animals were assigned randomly by computer program to the test groups in Table 1.

TABLE 1: STUDY DESIGN[a]

Test group	Nominal Conc. (g/M^3)	Analytical Conc. (g/M^3)[b]	MMAD μM	GSD μM	Rats/sex
Control	0	0.0±0.01[d]	--[c]	--[c]	5
Low (LCT)	0.5	0.54±0.04	5.0±0.5	1.6±0.3	5
Mid (MCT)	1.5	1.7±0.15	4.9±0.1	1.3±0.0	5
High (HCT)	3.0	2.97±0.34	4.8±0.1	1.4±0.1	5

[a]Data extracted from page 10 of this submission (MRID 41368823).
[b]Time-weighted average
[c]Insufficient number of particles for determination
[d]Mean and standard deviation from the mean

2. **Generation of the test atmosphere and description of the chamber:** For each group, animals (10/group) were jointly exposed to Light Neutral Oil, Gulf or air or 6 hours/day for 5 days in a 0.26 M^3 stainless steel and glass whole-body dynamic exposure chamber. Exposure chambers were maintained at a slight negative pressure (-0.04 to -0.05 water column inches) during exposures, and exposure start time was calculated as the time when exposure concentration neared the target dose level (the t90--time after generation startup that it take to reach 90% of the theoretical concentration). Test substance atmospheres were generated using a Solosphere nebulizer (McGraw Respiratory Therapy), and concentration of test substance was controlled by varying the amount of added air and inlet air pressure into the nebulizer. Atmospheres were passed through a condensing flask before entering the exposure chamber to eliminate large diameter particles.

Test atmosphere concentration: Particulates in exposure atmospheres were determined gravimetrically. Total particulate plus vapor concentrations were determined by collecting samples in cyclohexane behind fritted glass impingers and analysis by gas-liquid chromatography and comparison with known standards. Vapor phase concentrations were calculated by subtracting the particulate concentration from the total concentration.

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Total concentration results are presented in Table 1 above.

Particle size determination: An aerodynamic particle sizer (TSI, Inc., Model APS 33) was used to determine particle size once daily in terms of mass median aerodynamic diameter (MMAD) with the geometric standard deviation (GSD). Results are presented in Table 1 above.

3. Statistics - The means and standard deviations from the mean were calculated for all parameters investigated. Differences between group mean body weights were analyzed with Dunnett's test. No other statistical analyses were conducted.

C. METHODS:

1. Observations:

Animals were inspected twice daily on weekdays (dosing days) and once daily on weekends for mortality. Animals were observed once daily on dosing days only for clinical signs (immediately after exposure).

2. Body weight
Animals were weighed prior to the first exposure and at study termination on study day 5.

3. Food consumption

Food consumption was not measured.

4. Ophthalmoscopic examination

Eyes were not examined.

5. Blood was not collected or analyzed.

6. Urinalysis*

Urine was not analyzed.

* Not required for subchronic studies

7. Sacrifice and Pathology

Only the lungs from 2 mid-dose (1.70 g/M^3) females and 1 high-dose (2.97 g/M^3) male were examined microscopically.

II. RESULTS

A. Observations

1. Toxicity - Animals in the control group (0 g/M^3) exhibited no clinical signs, with the exception of perianal soiling in one female. All animals in the low-dose (0.54 g/M^3) group showed hair discoloration during study days 3-5; perianal soiling was apparent in 1/5 females during days 3-5; ocular porphyrin was observed in 1/5 females during days 4-5; ocular discharges were observed in 1/5 males at day 2 only; and dry red material around the mouth and/or nose was observed in 1/5 females at day 1 and in 2/5 males at day 3. All animals in the mid-dose (1.7 g/M^3) group exhibited discolored hair throughout the study; incidences of nasal discharges and red material around the mouth and/or nose were increased in both males and females during days 1-5; and ocular porphyrin was observed frequently in females throughout the study. In the high-dose (2.97 g/M^3) animals, respiratory effects (increased and/or labored respiration and harsh respiratory sounds) were observed in 1-4/5 females during study days 2-5; in addition, the incidences of the clinical signs observed in the mid-dose group were increased in incidence.

2. Mortality - Two females in the high-dose (2.97 g/M^3) group died unscheduled deaths; one died after exposure on day 2 and one died on day 4.

B. Body weight and weight gain A statistically significant decrease ($p \leq 0.05$; 88% control value) in mean body weight was seen in high-dose females (2.97 g/M^3) at Day 5. However, mean body weights never were less than 88% of the appropriate control values. Therefore, the biological significance of this small decrease is unknown. Representative body weight data are presented in the following Table 2:

Table 2. Body Weights (Grams) of Animals Exposed to Light Neutral Oil, Gulf for Five Days by Inhalation[a]

Study Day	Sex	0 (Control)	0.54 g/M^3	1.70 g/M^3	2.97 g/M^3
1	Male	291.60± 7.70[b] (100)[c]	291.05± 13.31 (100)	293.16± 8.06 (101)	291.50± 9.21 (100)
	Female	173.33± 10.95 (100)	169.31± 11.57 (98)	173.03± 5.34 (100)	172.21± 8.55 (99)

5	Male	290.04± 9.44 (100)	292.36± 11.84 (101)	291.60± 10.92 (101)	283.09± 10.39 (98)
	Female	174.18± 10.42 (100)	170.33± 12.21 (98)	168.51± 5.21 (97)	153.65± 9.41* (88)

[a]Data taken from page 20 of this submission (MRID 41368823).
[c]Mean and standard deviation from the mean.
[d]Figures in parentheses represent percentages of the appropriate control value.
*Indicates that $p \leq 0.05$.

C. Food consumption - Food consumption was not monitored.

D. Ophthalmoscopic examination - Eye examinations were not conducted.

E. Blood work was not conducted.

F. Urinalysis - Urinalyses were not conducted.

G. Sacrifice and pathology

1. Organ weights were not determined.

2. Gross pathology - High-dose (2.97 g/M^3) females exhibited abnormally colored contents in the duodenum (2/3), ileum (2/3), and jejunum (2/3). High-dose (2.97 g/M^3) males also exhibited abnormally colored contents in the duodenum (2.5), ileum (3/5), and jejunum (2/5). Mid-dose (1.70 g/M^3) males also exhibited abnormally colored contents in the ileum (2.5) and jejunum (2/5). Both high-dose (2.97 g/M^3) males (2/5) and females (1/3) exhibited abnormal liver color, and mid-dose (1.70 g/M^3) females also exhibited abnormal liver color (2/5).

3. Microscopic pathology

a) Non-neoplastic - Only the lungs from two mid-dose (1.70 g/M^3) females and one high-dose (2.97 g/M^3) male were examined microscopically. The lungs of the high-dose (2.97 g/M^3) male exhibited marked hyperplasia of alveolar macrophages and marked interstitial pneumonitis, as well as slight peribronchial lymphoid hyperplasia and slight perivascular (mononuclear) cuffing. Lungs of the two mid-dose (1.70 g/M^3) females exhibited moderate to severe hyperplasia of alveolar macrophages, severe interstitial pneumonitis, slight to moderate perivascular (mononuclear) cuffing, and none to moderate hemorrhage.

b) Neoplastic - No neoplastic lesions were observed in the study.

III. DISCUSSION

A. This study, which does not meet guideline 82-4 requirements for a subchronic (90-day) toxicity study in the rat, is nevertheless entirely acceptable for its intended purpose as a dose range-finding study. Because so few parameters were measured in this dose range-finding study, determination of a NOEL and LEL is problematic. However, from the data presented, **the LOEL is 1.70 g/M^3 (MCT; 1.70 mg/L), based on abnormal colored contents present in the ileum of males and abnormal lung histopathology (moderate to severe hyperplasia of alveolar macrophages, severe interstitial pneumonitis, slight to moderate perivascular (mononuclear) cuffing, and none to moderate hemorrhage) in females. The NOEL is 0.54 g/M^3 (0.54 mg/L; LCT).**

B. <u>Study deficiencies</u> This dose range-finding study has the following deficiencies with respect to guideline 82-4 for subchronic (90-day) inhalation toxicity in the rat: exposure was for five rather than ninety days; food consumption was not measured; no blood clinical chemistry or hematology studies were conducted; no ophthalmological examinations were conducted; no organs were weighed; and microscopic examination was limited to the lungs of three animals. These deficiencies cannot be remedied except by conducting a new study; however, this is not required since the study adequately serves as a dose range-finding study.

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100 Paraffine Oil, Gulf 14-Day Dermal Toxicity (82-2)

EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke, Date 8/22/96
Review Section 2, Toxicology Branch I (7509C) NSM for
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES, Date 8/26/96
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 14-Day Dermal Toxicity - Rabbit; OPPTS 870.3200, [§82-2]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): 100 Paraffine Oil, Gulf (No purity data)

SYNONYMS: None

CITATION: Zellers, J., and C. Whaley (1983) Two-week repeated dose toxicity study in rabbits using 100 paraffine oil. Gulf Life Sciences Center, Pittsburgh, PA. Report No.: 82-039, November 15, 1983. MRID 41368829. Unpublished.

SPONSOR: Gulf Life Sciences Center
Pittsburgh, PA

EXECUTIVE SUMMARY:

In a 14-day dermal toxicity study (MRID 41368829), 100 Paraffine Oil, Gulf (no purity data) was administered topically to the clipped backs (intact skin) of New Zealand White rabbits (3/sex/dose) at dose levels of 0, 1.0 or 2.0 grams/kg body weight/day for 5 days/week for a 2-week period. Three additional high-dose animals/sex were examined following a two-week recovery period, during which no treatment was administered. The vehicle (and control substance) was corn oil; test substance was administered neat for the high-dose (2.0 g/kg/day) group and as a 44.3% (w/v) solution in corn oil for the low-dose (1.0 g/kg/day) group. Dosing volume was maintained at approximately 2.258 ml/kg.

100 Paraffine Oil, Gulf elicited no biologically significant effects on body weight, food consumption, systemic (non-dermal) toxicity, blood hematology or clinical chemistry parameters, organ weights and organ/body weight or organ/brain weight ratios, or gross or microscopic findings at necropsy. Dermal reactions at the treatment site consisted of well defined to severe erythema, with slight to severe edema and eschar formation in the low-dose (1.0 g/kg/day) group; all of these reactions were more severe in the high-dose (2.0 g/kg/day) group and were confirmed with microscopic histopathology. Microscopic examination of skin from the application site of animals in the high-dose group

revealed epidermal acanthosis and hyperkeratosis, as well as excessive accumulation of keratin at the skin surface. **The systemic (non-dermal) LOEL is > 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The systemic NOEL is ≥ 2.0 g/kg/day. The dermal LOEL is < 1.0 g/kg/day (LDT), based increased incidence of edema and/or erythema and eschar formation at the treatment site with respect to controls (skin lesions were confirmed microscopically at 2.0 g/kg/day). The dermal NOEL is ≤ 1.0 g/kg/day (LDT).**

This 14-day dermal toxicity study is classified unacceptable, but ugradable to acceptable/non-guideline, with submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a 21-day dermal toxicity study (82-2) in the rabbit. However, this study provides useful information. Based on the lack of any toxicity other than dermal effects at the treatment site at doses up to approximately 2000 mg/kg/day, a repetition of this study is not required.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 100 Paraffine Oil, Gulf
   Description: Clear, amber-colored liquid
   Lot/Batch #: No data
   Purity: No data
   Stability of compound: No data
   CAS #: 64742-54-7

2. Vehicle and/or positive control: Corn Oil, Laboratory-Grade
   Purity: U.S.P.

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age and weight at study initiation: 12 weeks old; 2.401-2.879 kg body weight.

   Source: Dutchland Laboratories, Inc.
   Denver, PA
   Housing: Animals were housed individually in stainless steel cages.
   Diet: Certified Purina Rabbit Chow (#5322), ad libitum
   Water: Filtered tap water, ad libitum
   Environmental conditions:
   Temperature: 67.1±0.9°F.
   Humidity: 64.8±6.8%
   Air changes: Not reported
   Photoperiod: 12 hours

   Acclimation period: 3 weeks

B. STUDY DESIGN:

1. In life dates - start: 7/5/82 end: 7/19/82

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN: 14-Day Dermal Toxicity Study of 100 Paraffine Oil, Gulf in Rabbits[a]

Test Group	Dose to Animal (g/kg/day)	Male	Female
Vehicle Control (Corn Oil)	0.0	3	3
Low (LDT) (50.0% w/v in corn oil)	1.0	3	3
High (HDT) (Undiluted test substance)	2.0	3 (3)[b]	3 (3)[b]

[a]Data taken from page 9 of this submission (MRID 4136882906).
[b]These additional animals were examined and sacrificed after a two-week recovery period during which no treatment with 100 Paraffine Oil, Gulf was received.

Hair was clipped (electric clippers) from the backs of the test animals prior to study initiation. Animals were fitted with a neck collar to prevent ingestion of the test substance; this collar remained on each animal until sacrifice, except during weighing. Test solutions were applied topically to intact skin over approximately 10% of the body surface. Following application, the test sites were occluded with a polymer film-covered gauze patch. Trunks of the animals were wrapped with an elastic bandage. At six hours post-dosing, the treatment sites were exposed and excess test substance wiped from the skin. Doses were calculated based on individual body weights and adjusted weekly. Volumes of substances applied were approximately 2.3 mL. Treatments were conducted five days per week for a total of two weeks (3 days treatment; 2 days non-treatment; 5 days treatment; 2 days non-treatment; and 2 days treatment). High-dose

recovery animals were examined after a two-week recovery period, during which no treatments were administered.

3. Preparation and analysis of dosing solutions

Dilutions of test substance were prepared weekly. The corn oil vehicle and high-dose test substance (undiluted 100 Paraffine Oil, Gulf) were administered neat. No data are provided on the concentration, homogeneity, or stability of the test substance or its dilutions.

Results - No data were submitted.

4. Statistics - The following parameters were analyzed by group mean and standard deviation only: clinical chemistry and hematology values and absolute body and organ weight data. Additionally, relative organ weight data were analyzed by Barlett's test, and analysis of variance. If Barlett's test indicated homogeneous data, then Dunnett's test was conducted. If Bartlett's test indicated non-homogeneous data, then a modified t-test was employed. Incidences of microscopic findings were examined using the Kolmogorov-Smirnov two-tailed test.

C. METHODS:

1. Observations:

Animals were inspected twice daily on dosing days and once daily on non-dosing days for mortality and moribundity. Clinical signs were noted once daily on dosing days. Dermal reactions were scored (using a Draize method) twice daily on dosing days: once before dose application and once after removal of the occluding materials at 6 hours of exposure.

2. Body weight

Main-study animals were weighed prior to study initiation, weekly thereafter, and at study termination. Recovery group animals were additionally weighed at the end of the two-week recovery period.

3. Food consumption

Food consumption for each animal was determined daily on each dosing day.

4. Ophthalmoscopic examination

Examinations were not conducted.

5. Blood was collected via the auricular vessels for hematology and clinical analysis from all animals (NOT fasted overnight) prior to study initiation and at main study termination (for recovery animals, blood was additionally collected at sacrifice after two weeks of no treatment). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		Red cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
			Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase	X	Albumin/globulin ratio
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was not collected or analyzed.

* Not required for subchronic studies

7. Sacrifice and Pathology

One high-dose (2.0 g/kg/day) male suffered an unscheduled death; autopsy showed multiple duodenal and gastric ulcers unrelated to treatment. Animals sacrificed on schedule were subjected to gross pathological examination

and the CHECKED (X) tissues were collected from all animals for possible histological examination. Tissues were examined for control (0 g/kg/day) and high-dose (2.0 g/kg/day) animals. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*	XX	Brain*
	Salivary glands*	XX	Heart*		Periph. nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)[T]
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*	XX	Spleen*		Eyes (optic n.)[T]
	Jejunum*		Thymus*		
	Ileum*				
	Cecum*		UROGENITAL		GLANDULAR
	Colon*	XX	Kidneys*+		Adrenal gland*
	Rectum*		Urinary bladder*		Lacrimal gland[T]
XX	Liver*[+]	XX	Testes*[+]		Mammary gland[T]
	Gall bladder*		Epididymides		Parathyroids*[++]
	Pancreas*		Prostate		Thyroids*[++]
			Seminal vesicle		
	RESPIRATORY	XX	Ovaries		OTHER
	Trachea*		Uterus*		Bone
	Lung*				Skeletal muscle
	Nose			X	Skin
	Pharynx				All gross lesions and masses*
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
[+] Organ weight required in subchronic and chronic studies.
[++] Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations :

1. Toxicity - With the exception of dermal reactions, the incidences of clinical signs in treated animals were similar to those observed in appropriate controls. In the 1 g/kg/day group, all animals exhibited well-defined erythema during the course of the study, reaching the severe stage in one male. Eighty-three percent of the animals in this group exhibited moderately severe edema, 33% had fissures and/or eschar formation, and the remainder exhibited well-defined edema. In the 2 g/kg/day group, all animals exhibited moderate edema, 83% had fissures and/or eschar at the test site, and 11/12 showed moderate to severe erythema.

2. Mortality - One high-dose (2.0 g/kg/day) male died during the two-week recovery period; autopsy showed multiple

gastric and duodenal ulcers unrelated to treatment. All remaining animals survived until scheduled sacrifice.

B. Body weight: As shown in Table 2, although females in the high-dose (2.0 g/kg/day) group exhibited slight decreases, there were no biologically significant treatment-related effects on body weight.

Table 2. Body Weights (Kilograms) of Rabbits Treated Dermally for 14 Days with 100 Paraffine Oil, Gulf[a]

Dose (g/kg/day)	Sex	Study Day -1	Study Day 7	Study Day 14	Study Day 28
0 (Corn Oil Control)	Male	2.6230± 0.1176[b] (100)[c]	2.4970± 0.1025 (100)	2.5713± 0.1324 (100)	--
	Female	2.7040± 0.1730 (100)	2.6950± 0.2986 (100)	2.7843± 0.3086 (100)	--
1.0 (Low Dose) (LDT)	Male	2.5667± 0.1241 (98)	2.5047± 0.1309 (100)	2.5563± 0.0852 (99)	--
	Female	2.5513± 0.1591 (94)	2.4297± 0.3215 (90)	2.5340± 0.2022 (91)	--
2.0 (High Dose) (HDT)	Male	2.7260± 0.0275 (104)	2.5420± 0.0131 (102)	2.5777± 0.0307 (93)	--
	Female	2.6790± 0.1100 (99)	2.5457± 0.2307 (94)	2.4697± 0.2619 (89)	--
2.0 (After 2-Week Recovery)	Male	2.5950± 0.0679 (100)[d]	2.4625± 0.0064 (95)	2.482.0± 0.0099 (96)	2.7845± 0.0389 (107)
	Female	2.4767± 0.0994 (100)	2.3960± 0.1485 (97)	2.3683± 0.1219 (96)	2.5040± 0.02164 (101)

[a]Data extracted from pages 24-27 of this submission (MRID 41368829).
[b]Mean ± standard deviation from the mean.
[c]Figures in parentheses indicate percentages of appropriate control values.
[d]For recovery animals, the day -1 body weight has been set as 100% (internal control value).

C. Food consumption

1. Food consumption - There were no biologically significant treatment-related effects on food consumption.

2. Food efficiency - Food efficiency was not determined.

D. Ophthalmoscopic examination - Ocular examinations were not conducted.

E. Blood work:

1. Hematology - The hematological values observed for animals treated with 100 Paraffine Oil, Gulf, at both dose levels tested, were comparable to those obtained for the corn oil control animals.

2. Clinical Chemistry - The clinical chemistry values obtained for animals treated with 100 Paraffine Oil, Gulf, at both dose levels were comparable to those exhibited by animals in the corn oil control group.

F. Urinalysis - No urinalyses were conducted.

G. Sacrifice and Pathology:

1. Organ weight - Organ weights and organ/body weight and organ/brain weight ratios were comparable to appropriate control values for the low-dose (1.0 g/kg/day) and high-dose (2.0 g/kg/day) group animals sacrificed after two weeks, as well as for the high-dose (2.0 g/kg/day) group sacrificed after two weeks of recovery (during which no 100 Paraffine Oil, Gulf was administered.

2. Gross pathology - No treatment-related lesions were observed at gross necropsy. One male in the control (0 g/kg/day) and one female in the high-dose (2.0 g/kg/day) recovery group (sacrificed after a two-week recovery period following 14 days of treatment) exhibited focal areas of thickening of the skin or erythema at the application site. One male in the high-dose (2.0 g/kg/day) recovery group died on test; autopsy revealed multiple duodenal and stomach ulcers.

3. Microscopic pathology -

a) Non-neoplastic - The only treatment-related microscopic lesions observed consisted of epidermal acanthosis and hyperkeratosis of the skin at the treatment site in high-dose (2.0 g/kg/day) males and females. Thickening of the epidermis occurred, due to hyperplasia of the basilar epithelium and keratin accumulation on the skin surface.

Treatment-unrelated lesions were consistent with an infection by the protozoan parasite, Encephalitozoon cuniculi, or were those normally observed as a result of common diseases in the New Zealand White rabbit.

b) Neoplastic - No treatment-related neoplastic lesions were detected.

III. DISCUSSION

A. The only biologically significant effects observed following the dermal administration of 100 Paraffine Oil, Gulf to New Zealand White rabbits were histologically confirmed skin lesions at the treatment site. Although the testing facility noted that body weights were generally decreased in females in the high-dose (2.0 g/kg/day) group, mean body weight for this group at 14-day sacrifice represented 89% of control value (greatest decrease observed), and these decreases are therefore not considered to be biologically significant. The **systemic LEL is > 2.0 g/kg/day (HDT), based on the lack of any systemic toxicity. The systemic NOEL is ≥ 2.0 g/k/g/day (HDT). The dermal LEL is < 1.0 g/kg/day (LDT), based on well defined erythema edema, and eschar formation at 1.0 g/kg/day and microscopic confirmation of dermal effects in the high-dose (2.0 g/kg/day) group. The dermal NOEL is ≤ 1.0 g/kg/day (LDT).** This study has been classified unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance. However, based on the lack of systemic toxicity at dose levels up to 2.0 g/kg/day, requiring a repetition of this study would not yield any additional meaningful toxicological information on this substance.

B. Study deficiencies - No data were submitted on the purity or stability of the test substance. Dosing was conducted 5 days/week for a total of 2 weeks rather than the guideline procedure of at least 5 days/week for a total of 3 weeks. The following clinical chemistry blood parameters were not determined: calcium, chloride, phosphorus, and SGOT. In addition, no blood clotting measurement was determined during hematology analyses. Only very limited gross and microscopic examinations of organs were conducted.

012030

EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke Date 8/22/96
Review Section 2, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES, Date 8/26/96
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 5-Day Dermal Toxicity - Rat; OPPTS 870.3200, [§82-2]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): Light Neutral Oil, Gulf (No purity data)

SYNONYMS: None

CITATION: Zellers, J., and D. Meckley (1984) Five-day repeated dose dermal toxicity study in rats of light neutral oil. Gulf Life Sciences Center, Pittsburgh, PA. Report No.: 1184, April 12, 1984. MRID 41368821. Unpublished.

SPONSOR: Gulf Life Sciences Center
Pittsburgh, PA

EXECUTIVE SUMMARY:

In a 5-day dermal toxicity study (MRID 41368821), Light Neutral Oil, Gulf (no purity data) was administered topically to the clipped backs (intact skin) of Fischer 344 rats (5/sex/dose) at dose levels of 0, 0.85, 1.0 or 2.0 grams/kg body weight/day for 5 days/week for a one-week period (two days of treatment; two days of non-treatment; three days of treatment). The vehicle (and control substance) was heavy paraffin oil; test substance was administered neat for the high-dose (2.0 g/kg/day) and mid-dose (1.0 g/kg/day) groups and as a 42.5% (w/v) solution in heavy paraffin oil for the low-dose (0.85 g/kg/day) group.

Light neutral oil, Gulf elicited no biologically significant effects on survival, clinical or toxicological signs, body weight, food consumption, systemic toxicity, dermal toxicity, or findings at gross necropsy. The **systemic and dermal NOEL ≥ 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The LOEL is > 2.0 g/kg/day.**

This 5-day dermal toxicity study is classified unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance. However, this study provides useful information. Based on the lack of any toxicity at doses up to approximately 2000 mg/kg/day, a repetition of this study is not required.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Light Neutral Oil, Gulf
   Description: Clear, colorless liquid
   Lot/Batch #: No data
   Purity: No data
   Stability of compound: No data
   CAS #: Not provided

2. Vehicle and/or positive control: Heavy Paraffin Oil, Laboratory-Grade
   Purity: U.S.P.

3. Test animals: Species: Rat
   Strain: Fischer 344
   Age and weight at study initiation: 54(M) and 59(F) days old; 114.6-165.7 g body weight.

   Source: Charles River Breeding Laboratories, Inc. Portage, MI
   Housing: Animals were housed individually in stainless steel cages.
   Diet: Certified Purina Rat Chow (#5002), ad libitum
   Water: Filtered tap water, ad libitum
   Environmental conditions:
   Temperature: 77.6°F (mean)
   Humidity: 63.7% (mean)
   Air changes: Not reported
   Photoperiod: 12 hours

   Acclimation period: 1 week

B. STUDY DESIGN:

1. In life dates - start: 8/25/83 end: 8/30/83

2. Animal assignment

   Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN: 5-Day Dermal Toxicity Study of Light Neutral Oil, Gulf in Rats[a]

Test Group	Dose to Animal (g/kg/day)	Male	Female
Vehicle Control (Heavy paraffin oil)	0.0	5	5
Low (LDT) (42.5% w/v in heavy paraffin oil)	0.85	5	5
Mid (MDT) (Undiluted test substance)	1.0	5	5
High (HDT) (Undiluted test substance)	2.0	5	5

[a]Data taken from page 5 of this submission (MRID 41368821).

Hair was clipped (electric clippers) from the backs of the test animals prior to study initiation. Each test group contained 5 males and 5 females. Animals were fitted with a neck collar just before dosing to prevent ingestion of the test substance; this collar remained on each animal during the 6-hour exposure period. Test solutions were applied topically over approximately 10% of the body surface. At six hours post-dosing, the excess test substance was wiped from the skin. Doses were calculated based on individual body weights. Volumes of substances applied varied from 1.8 to 2.36 mL/kg of body weight Treatments were conducted five days per week for a total of one week [two days of treatment; two days of no treatment (weekend); three days of treatment].

3. Preparation and analysis of dosing solutions

The 42.5% (w/v) dilution of the test substance was prepared on the day of study initiation and used throughout the study. The heavy paraffin oil vehicle and high-dose test substance (undiluted light neutral oil,

Gulf) were administered neat. No data are provided on the concentration, homogeneity, or purity of the test substance or its dilution.

Results - No data were submitted.

4. Statistics - Food consumption and body weight data were analyzed by mean and standard deviation, Barlett's test, and analysis of variance. If Barlett's test indicated homogeneous data, then Dunnett's test was conducted. If Bartlett's test indicated non-homogeneous data, then a modified t-test was employed.

C. METHODS:

1. Observations:

Animals were inspected twice daily on dosing days and once daily on non-dosing days for mortality and moribundity. Clinical signs were noted once daily on dosing days. Dermal reactions were scored (using a Draize method) prior to the first treatment, daily on dosing days after removal of excess test material after 6 hours of exposure, and prior to sacrifice.

2. Body weight

Animals were weighed prior to study initiation and at study termination.

3. Food consumption

Food consumption for each animal was determined over the entire 7-day period.

4. Sacrifice and Pathology

All animals that suffered unscheduled deaths (none in this study) and those sacrificed on schedule were subjected to gross pathological examination. However, the organs examined were not identified and gross pathology findings listed only organs for which findings were identified. No organs were weighed or collected for current or future histopathology.

II. RESULTS

A. Observations :

1. Toxicity - No clinical signs attributable to treatment were observed. Treatment-unrelated observations included nasal and ocular discharges in all treatment groups,

including the vehicle control groups. No dermal reactions were noted in any animal on test.

2. Mortality - There were no unscheduled deaths; all animals survived to study termination. No moribund animals were observed.

B. Body weight: As shown in Table 2, there were no biologically significant treatment-related effects on body weight.

Table 2. Body Weights (Grams) of Animals Treated Dermally for Seven Days with Light Neutral Oil, Gulf[a]

Dose (g/kg/day)	Sex	Terminal Body Weight (g)
0 (Heavy Mineral Oil Control)	Male	174.46± 15.36[b] (100)[c]
	Female	132.02± 5.20 (100)
0.85 (Low Dose; LDT)	Male	174.60± 9.31 (100)
	Female	132.08± 7.20 (100)
1.0 (Mid Dose; MDT)	Male	168.78± 9.33 (97)
	Female	133.86± 1.91 (101)
2.0 (High Dose; HDT)	Male	172.60± 5.92 (99)
	Female	134.38± 10.06 (102)

[a]Data extracted from page 19 of this submission (MRID 41368821).
[b]Mean ± standard deviation from the mean.
[c]Figures in parentheses indicate percentages of appropriate control values.

C. Food consumption

1. Food consumption - As shown in Table 3, there were no biologically significant treatment-related effects on food consumption for any females on test. Food consumption for treated males was generally decreased, and statistically significantly so for the males in the 2.0 g/kg/day group. However, this decrease in food consumption was not reflected in lower body weight, and the biological significance of this decrease is therefore unknown.

Table 3. Mean Food Consumption (g/animal/day) of Animals Treated Dermally for Seven Days with Light Neutral Oil, Gulf[a]

Dose (g/kg/day)	Sex	Mean Food Consumption
0 (Heavy Mineral Oil Control)	Male	133.24± 7.24[b] (100)[c]
	Female	94.70± 0.52 (100)
0.85 (Low Dose; LDT)	Male	127.08± 6.97 (95)
	Female	93.52± 3.05 (110)
1.0 (Mid Dose; MDT)	Male	125.02± 4.98 (94)
	Female	93.92± 5.35 (99)
2.0 (High Dose; HDT)	Male	122.16± 1.85* (92)
	Female	94.68± 3.07 (100)

[a]Data extracted from page 32 of this submission (MRID 41368821).
[b]Mean ± standard deviation from the mean.
[c]Figures in parentheses indicate percentages of

appropriate control values.
*Indicates that $p \leq 0.05$.

2. Food efficiency - Food efficiency was not determined.

D. Ophthalmoscopic examination - Ocular examinations were not conducted.

E. Blood work: Blood work was not performed.

F. Urinalysis - No urinalyses were conducted.

G. Sacrifice and Pathology:

1. Organ weight - Organ weights were not determined.

2. Gross pathology - No treatment-related lesions were observed at gross necropsy. Treatment-unrelated findings included linear corneal opacity in one high-dose (2.0 g/kg/day) male and miscellaneous findings in controls and all treatment groups generally observed in Fischer 344 rats.

3. Microscopic pathology - No microscopic histopathology was performed.

III. DISCUSSION

Light neutral oil, Gulf elicited no dermal or systemic toxicity when applied topically to the clipped backs of Fischer 344 rats at doses up to 2.0 g/kg body weight/day over a 7-day period during which 5 doses were administered. Therefore, the **systemic and dermal NOEL ≥ 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The LOEL is > 2.0 g/kg/day.** This study has been classified as unacceptable, but upgradable; however, based on the lack of any apparent toxicity at high doses, requiring a repetition of this study would not yield any meaningful additional toxicity information about this substance.

B. Study deficiencies - No data were submitted on the purity or stability of the test substance. Dosing was conducted 5 days/week for a total of one week rather than the 82-2 guideline procedure of at least 5 days/week for a total of 3 weeks. In addition, no blood work was performed (hematology and clinical chemistry), no organs were weighed, and no histopathological examination of tissues was conducted.

012030

EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke, Date 8/12/96
Review Section 2, Toxicology Branch I (7509C) (MSmith)
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES, Date 8/26/96
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - Rat; OPPTS 870.3465 [§82-4]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): 70 Orchard Spray (No purity data)

SYNONYMS: None

CITATION: Gordon, T. and F. Steele (1983) Nine day repeated dose inhalation toxicity study in rats - 70 Orchard Spray. Gulf Life Sciences Center, Pittsburgh, PA. Report Number 82-064, September 15, 1983. MRID 41368807. Unpublished.

SPONSOR: Gulf Oil Refining and Marketing Company Houston, TX

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 41368807), 70 Orchard Spray (no purity data) was administered to Fischer 344 rats (5/sex/dose) by whole body inhalation exposure at analytically determined concentrations of 0.0, 0.70, or 1.60 g/M^3 (equivalent to 0.0, 0.70, or 1.60 mg/L) for six hours per day, for a total of nine exposures.

No biologically significant effects were noted on body weight, hematology parameters, or blood clinical chemistry values. One high-dose (1.60 g/M^3) female died a treatment-related (lung effects) unscheduled death after three exposures. Dose-related clinical signs included test substance on the fur, ocular porphyrin discharges, nasal discharges, and dry red discharges around the nose and/or mouth, perianal soiling, excessive tearing, and closed eyes. High-dose females also exhibited episodes of labored breathing. 70 Orchard Spray elicited statistically significant increases in absolute weight of the lungs for males (135% control) and females (137% control) in the high-dose (1.6 g/M^3) group, and for females in the low-dose group (0.7 g/M^3; 137% control). High-dose (1.6 g/M^3) males exhibited statistically significantly increased (133% control) absolute liver weight, liver/body weight ratio (114% control), liver/brain weight ratio (112% control), lung/body weight ratio (135% control), and lung/brain weight ratio (132% control). High-dose (1.6 g/M^3) females exhibited statistically significantly

increased liver/body (116% control) and lung/body (150% control) weight ratios and lung/brain (139% control) weight ratios. Low-dose (0.70 g/M^3) females exhibited increased lung/body (144% control) and lung/brain (141% control) weight ratios. The incidence of microscopically identified hypertrophy and hyperplasia of the alveolar macrophages of the lung was statistically significant ($p \leq 0.05$) for both males and females in the high-dose (1.6 g/M^3) group (5/5 for both sexes). Perivascular/bronchial edema was also observed in both males (2/5) and females (4/5) in the high-dose group, but was statistically significant ($p \leq 0.05$) only for the females. These lesions were especially severe in the one high-dose female that died an unscheduled death. From the data presented, **the LOEL is ≤ 0.70 g/M^3 (LCT; 0.70 mg/mL), based on increased absolute weight of lungs (137% control) and lung/body (144% control) and lung/brain (141% control) weight ratios in females.** Lung lesions were observed microscopically in both males and females in the high-dose (1.60 g/M^3) group, but no microscopic examination was conducted on tissues from low-dose (0.70 g/M^3) animals. **The NOEL < 0.70 g/M^3.**

This subchronic toxicity study is unacceptable, but upgradable to acceptable/non-guideline, with the submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a subchronic inhalation study (82-4) in the rat. However, this study is entirely satisfactory for its intended use as a dose range-finding study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 70 Orchard Spray
   Description: Clear, colorless oil
   Lot/Batch #: Not given
   Purity: Not given
   Stability of compound: Not given
   CAS #: 64742-55-8

2. Vehicle and/or positive control: None used
   Lot/Batch # None

3. Test animals: Species: Rat
   Strain: Fischer 344
   Age and weight at study initiation: 10 weeks; 120-208 g
   Source: Charles River Breeding Laboratories, Inc.
   Kingston, NY
   Housing: Individually in stainless steel cages
   Diet: Purina Certified Rodent Diet #5002, ad libitum (except during exposure)

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Water: Tap water, ad libitum
Environmental conditions: Temperature: 75±1°F
Humidity: 48±3%
Air changes: Not reported
Photoperiod: 12 hours dark/light
Acclimation period: Time period not given

B. STUDY DESIGN:

1. In life dates - start: 10/25/82 end: 11/5/82

2. Animal assignment

Animals were assigned randomly by computer program to the test groups in Table 1.

TABLE 1: STUDY DESIGN[a]

Test group	Nominal Conc. (g/M^3)	Analytical Conc. (g/M^3)[b]	MMAD μM	GSD μM	Rats/sex
Control	0	Below detection limits	--[c]	--[c]	5
Low (LCT)	0.5	0.7±0.1[d]	4.4±1.7	2.8±0.7	5
High (HCT)	1.5	1.6±0.3	4.8±0.9	2.8±0.4	5

[a]Data extracted from page 6 of this submission (MRID 41368807).
[b]Time-weighted average
[c]Insufficient number of particles for determination
[d]Mean and standard deviation from the mean

2. **Generation of the test atmosphere and description of the chamber:** For each group, animals (10/group) were jointly exposed to 70 Orchard Spray or air or 6 hours/day for 9 days in a 0.26 M^3 stainless steel and glass whole-body dynamic exposure chamber. Exposure chambers were maintained at a slight negative pressure (-0.03 to -0.07 water column inches) during exposures, and exposure start time was calculated as the time when exposure concentration neared the target dose level (the t95--time after generation startup that it take to reach 95% of the theoretical concentration--was approximately 29 minutes). Test substance atmospheres were generated using a Solosphere nebulizer (McGraw Respiratory Therapy), and concentration of test substance was controlled by varying the amount of added air and inlet air pressure into the nebulizer. Atmospheres were passed through a condensing flask before entering the exposure chamber to eliminate large diameter particles.

Test atmosphere concentration: Particulates in exposure atmospheres were determined gravimetrically. Total particulate plus vapor concentrations were determined by collecting samples in cyclohexane behind fritted glass impingers and analysis by gas-liquid chromatography and comparison with known standards. Vapor phase concentrations were calculated by subtracting the particulate concentration from the total concentration. Total concentration results are presented in Table 1 above.

Particle size determination: An aerodynamic particle sizer (TSI, Inc., Model APS 33) was used to determine particle size once daily in terms of mass median aerodynamic diameter (MMAD) with the geometric standard deviation (GSD). Results are presented in Table 1 above.

3. Statistics - The means and standard deviations from the mean were calculated for all parameters investigated. Differences between group mean body weights were analyzed with Dunnett's test. For clinical chemistry and hematology values, Barlett's test and analysis of variance were performed; if homogeneity was indicated, Dunnett's test was performed; if nonhomogeneity was indicated, a modified t-test was conducted. Organ weight data were analyzed in the same manner as clinical chemistry and hematology values, except that an ANOVA test was additionally conducted. Microscopic findings were analyzed by a two-tailed Kolmogorov-Smirnov analysis.

C. METHODS:

1. Observations:

Animals were inspected twice daily on weekdays (dosing days) and once daily on weekends for mortality. Animals were observed once daily on dosing days only for clinical signs.

2. Body weight
Animals were weighed prior to the first exposure and on days 7, and 12 (prior to sacrifice).

3. Food consumption

Food consumption was not measured.

4. Ophthalmoscopic examination

Eyes were not examined.

5. Blood was collected from non-fasted, anesthetized animals via the orbital sinuses for hematology and clinical analysis (from all animals at terminal sacrifice). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
	Potassium*		Globulins
	Sodium*	X	Glucose*
			Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase	X	Albumin/globulin ratio
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was not analyzed.

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the

CHECKED (X) tissues were collected for histological examination. Only the underlined organs were examined for the high-dose (1.6 g/M^3) and control (0.0 g/M^3) groups only. The (XX) organs, in addition, were weighed, except for those from the high-dose female that died on test (organs from this female were examined microscopically, however).

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*	XX	Brain*
	Salivary glands*	XX	Heart*		Periph. nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)^T (lumbar)
	Stomach*		Lymph nodes* (mesenteric)		Pituitary*
	Duodenum*	XX	Spleen*		Eyes (optic n.)^T
	Jejunum*		Thymus*		
	Ileum*				
	Cecum*		UROGENITAL		GLANDULAR
	Colon*	XX	Kidneys*+		Adrenal gland*
	Rectum*		Urinary bladder*		Lacrimal gland^T
XX	Liver*+	XX	Testes*+		Mammary gland^T
	Gall bladder*		Epididymides		Parathyroids*++
	Pancreas*		Prostate		Thyroids*++
	RESPIRATORY		Seminal vesicle		
	Trachea*		Ovaries		OTHER
XX	Lung*		Uterus*		Bone
	Pharynx		Vagina		Skeletal muscle
	Larynx				Skin
					All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines.
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ.
++ Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. Toxicity - Animals in the control group exhibited only sporadic incidences of clear nasal discharge and dry red material around the nose and mouth. Animals in the low-dose (0.7 g/M^3) group showed increased incidences of the control group clinical signs as well as excessive tearing (almost all animals from Day 4 on) and closed eyes (all animals at Day 9). High-dose (1.6 g/M^3) animals exhibited greatly increased incidences of the control group signs; all animals showed discharges from the eyes and ocular porphyrin from Day 4 on, 4/5 females exhibited labored breathing at Day 2 and 1/4 continued to exhibit this effect during Days 3-4; perianal soiling was noted

in 3/5 females at Day 2; and hair discoloration was noted in all animals in this group after Day 5.

2. Mortality - All animals survived until scheduled sacrifice, with the exception of one high-dose (1.6 g/M^3) female that died after the third exposure.

B. Body weight and weight gain A statistically significant decrease ($p \leq 0.05$; 95% control value) in mean body weight was seen in high-dose females (1.60 g/M^3) at Day 7, but this effect was not seen at Day 12. Furthermore, mean body weights never were less than 88% of the appropriate control values. Therefore, the biological significance of these small decreases is unknown. Representative body weight data are presented in the following Table 2:

Table 2. Body Weights of Animals Exposed to 70 Orchard Spray for Nine Days by Inhalation[a,b]

Study Day	Sex	0 (Control)	0.70 g/M^3	1.60 g/M^3
1	Male	193.17± 14.18[c] (100)[d]	193.73± 14.36 (100)	202.68± 8.17 (105)
	Female	136.08± 8.90 (100)	131.69± 5.80 (97)	120.30± 8.18 (88)
7	Male	207.63± 11.41 (100)	207.06± 8.14 (100)	203.31± 5.63 (98)
	Female	142.43± 8.04 (100)	134.26± 8.30 (94)	134.93± 9.53* (95)
12	Male	221.74± 10.36 (100)	220.87± 7.63 (100)	220.78± 9.06 (100)
	Female	149.96± 4.31 (100)	142.89± 8.29 (95)	137.92± 8.66 (92)

[a]Data taken from page 19 of this submission (MRID 41368807).
[b]Submitted data were almost illegible; therefore the figures presented in Table 2 represent the reviewing toxicologist's best estimate.
[c]Mean and standard deviation from the mean.
[d]Figures in parentheses represent percentages of the appropriate control value.
*Indicates that $p \leq 0.05$.

C. Food consumption - Food consumption was not monitored.

D. Ophthalmoscopic examination - Eye examinations were not conducted.

E. Blood work

1. Hematology - Hematological values for all treated animals were comparable to the appropriate control values.

2. Clinical Chemistry - Clinical chemistry values for all treated animals were comparable to the appropriate control values.

F. Urinalysis - Urinalyses were not conducted.

G. Sacrifice and pathology

1. Organ weight - 70 Orchard Spray elicited statistically significant increases in absolute weight of the lungs for males (135% control) and females (137% control) in the high-dose (1.6 g/M^3) group, and for females in the low-dose group (0.7 g/M^3; 137% control). High-dose (1.6 g/M^3) males exhibited statistically significantly increased (133% control) absolute liver weight, liver/body weight ratio (114% control), liver/brain weight ratio (112% control), lung/body weight ratio (135% control), and lung/brain weight ratio (132% control). High-dose (1.6 g/M^3) females exhibited statistically significantly increased liver/body (116% control) and lung/body (150% control) weight ratios and lung/brain (139% control) weight ratios. Low-dose (0.70 g/M^3) females exhibited increased lung/body (144% control) and lung/brain (141% control) weight ratios. These data are presented in the following Tables 3, 4, and 5.

Table 3. Absolute Organ Weights of Organs from Animals Exposed to 70 Orchard Spray for Nine Days by Inhalation[a]

Organ	Sex	0 (Control)	0.70 g/M^3	1.60 g/M^3
Lungs	Male	1.0914± 0.1686[b] (100)[c]	1.3554± 0.2499 (124)	1.4694± 0.2180* (135)
	Female	0.8336± 0.0605 (100)	1.1422± 0.0853** (137)	1.1453± 0.0713** (137)
Liver	Male	9.6386± 0.5152 (100)	10.3832 1.0044 (108)	10.9676 0.3164* (133)

Liver	Female	5.9782± 0.5038 (100)	6.1960± 0.7909 (104)	6.3873± 0.3593 (107)

[a]Data taken from page 31 of this submission (MRID 41368807).
[b]Means and standard deviations from the means.
[c]Numbers in parentheses represent percentages of the appropriate control values.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

Table 4. Organ/Body Weight Ratios of Animals Exposed to 70 Orchard Spray for Nine Days by Inhalation[a]

Organ	Sex	0 (Control)	0.70 g/M^3	1.60 g/M^3
Lungs	Male	0.4920± 0.0700 (100)	0.6084± 0.1132 (124)	0.6655± 0.0963* (135)
	Female	0.5576± 0.0260 (100)	0.8029± 0.1019** (144)	0.8343± 0.0944** (150)
Liver	Male	4.3484± 0.1666[b] (100)[c]	4.6524± 0.3208 (107)	4.9686± 0.1336** (114)
	Female	3.9912± 0.1579 (100)	4.3203± 0.3311 (108)	4.6414± 0.3226** (116)

[a]Data taken from page 33 of this submission (MRID 41368807).
[b]Mean and standard deviation from the mean.
[c]Figures in parentheses represent percentages of the appropriate control value.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

Table 5. Organ/Brain Weight Ratios of Animals Exposed to 70 Orchard Spray for Nine Days by Inhalation[a]

Organ	Sex	0 (Control)	0.70 g/M^3	1.60 g/M^3
Lungs	Male	0.6363± 0.0929 (100)	0.7883± 0.1348 (124)	0.8411± 0.1292* (132)
	Female	0.5033± 0.0350 (100)	0.7114± 0.0558** (141)	0.7020± 0.0587** (139)

Liver	Male	5.6230± 0.2600[b] (100)[c]	6.0509± 0.6198 (108)	6.2734± 0.1898* (112)
	Female	3.6080± 0.3226 (100)	3.8570± 0.4817 (107)	3.9100± 0.1932 (108)

[a]Data taken from page 35 of this submission (MRID 41368807).
[b]Mean and standard deviation from the mean.
[c]Figures in parentheses represent percentages of the appropriate control value.
*Indicates that $p \leq 0.05$.
**Indicates that $p \leq 0.01$.

2. Gross pathology - One high-dose (1.6 g/M^3) female exhibited lung congestion and fluid in the nasal passages. No other treatment-related lesions were observed.

3. Microscopic pathology

a) Non-neoplastic - The incidence of microscopically identified hypertrophy and hyperplasia of the alveolar macrophages of the lung was statistically significant ($p \leq 0.05$) for both males and females in the high-dose (1.6 g/M^3) group (5/5 for both sexes). Perivascular/bronchial edema was also observed in both males (2/5) and females (4/5) in the high-dose group, but was statistically significant ($p \leq 0.05$) only for the females. These lesions were especially severe in the one high-dose female that died an unscheduled death.

b) Neoplastic - No neoplastic lesions were observed in the study.

III. DISCUSSION

A. This study, which does not meet guideline 82-4 requirements for a subchronic (90-day) toxicity study in the rat, is nevertheless entirely acceptable for its intended purpose as a dose range-finding study. From the data presented, **the LOEL is ≤ 0.70 g/M^3 (LCT; 0.70 mg/mL), based on increased absolute weight of lungs (137% control) and lung/body (144% control) and lung/brain (141% control) weight ratios in females.** Lung lesions were observed microscopically in both males and females in the high-dose (1.60 g/M^3) group, but no microscopic examination was conducted on tissues from low-dose (0.70 g/M^3) animals. **The NOEL < 0.70 g/M^3.**

B. Study deficiencies This dose range-finding study has the following deficiencies with respect to guideline 82-4 for subchronic (90-day) inhalation toxicity in the rat: exposure

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was for nine rather than ninety days; no NOEL was established; food consumption was not measured; no measure of platelet count or blood-clotting potential was undertaken; no ophthalmological examinations were conducted; very few clinical chemistry measurements were included in the hematology aspect of the study; and very few organs were examined. These deficiencies cannot be remedied except by conducting a new study, which is not required since the study adequately serves as a dose range-finding study.

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EPA Reviewer: Raymond K. Locke, Toxicologist Raymond Locke, Date 8/12/96
Review Section 2, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES, Date 8/26/96
Section Head, Review Section 2, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 14-Day Dermal Toxicity - Rabbit; OPPTS 870.3200, [§82-2]

DP BARCODE: D217192 SUBMISSION CODE: S474639
P.C. CODE: 063503 TOX. CHEM. NO.: 646A

TEST MATERIAL (PURITY): Gulf Orchard Spray 70 (No purity data)

SYNONYMS: None

CITATION: Zellers, J., and D. Crutchfield (1983) Two-week repeated dose toxicity study in rabbits using Gulf Orchard Spray 70. Gulf Life Sciences Center, Pittsburgh, PA. Report No.: 82-046, June 15, 1983. MRID 41368806. Unpublished.

SPONSOR: Gulf Life Sciences Center
Pittsburgh, PA

EXECUTIVE SUMMARY:

In a 14-day dermal toxicity study (MRID 41368806), Gulf Orchard Spray 70 (no purity data) was administered topically to the clipped backs (intact skin) of New Zealand White rabbits (3/sex/dose) at dose levels of 0, 1.0 or 2.0 grams/kg body weight/day for 5 days/week for a 2-week period. Three additional high-dose animals/sex were examined following a two-week recovery period, during which no treatment was administered. The vehicle (and control substance) was corn oil; test substance was administered neat for the high-dose (2.0 g/kg/day) group and as a 43.1% (w/v) solution in corn oil for the low-dose (1.0 g/kg/day) group.

Gulf Orchard Spray 70 elicited no biologically significant effects on body weight, food consumption, systemic (non-dermal) toxicity, blood hematology or clinical chemistry parameters, organ weights and organ/body weight or organ/brain weight ratios, or gross or microscopic findings at necropsy. Dermal reactions at the treatment site consisted of slight edema and/or erythema in the low-dose (1.0 g/kg/day) group and much more severe erythema and edema, together with desquamation, in the high-dose (2.0 g/kg/day) group. These dermal observations were confirmed microscopically. Microscopic examination revealed epidermal acanthosis and hyperkeratosis, as well as excessive accumulation of keratin at the skin surface. **The systemic (non-dermal) LOEL**

is > 2.0 g/kg/day (HDT), based on the lack of any toxic effects. The systemic NOEL is ≥ 2.0 g/kg/day. The dermal LOEL is ≤ 1.0 g/kg/day (LDT), based increased incidence of edema and/or erythema at the treatment site with respect to controls (skin lesions were confirmed microscopically at 2.0 g/kg/day). The dermal NOEL is < 1.0 g/kg/day (LDT).

This 14-day dermal toxicity study is classified unacceptable, but upgradable to acceptable/non-guideline with the submission of adequate data on the purity and stability of the test substance; it does not satisfy the guideline requirement for a 21-day dermal toxicity study (82-2) in the rabbit. However, this study provides useful information. Based on the lack of any toxicity other than dermal effects at the treatment site at doses up to approximately 2000 mg/kg/day, a repetition of this study is not required.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Gulf Orchard Spray 70
   Description: Clear, colorless liquid
   Lot/Batch #: No data
   Purity: No data
   Stability of compound: No data
   CAS #: 64742-55-8

2. Vehicle and/or positive control: Corn Oil, Laboratory-Grade
   Purity: U.S.P.

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age and weight at study initiation: 13 weeks old; 2.406-3.072 kg body weight.

   Source: Dutchland Laboratories, Inc.
   Denver, PA
   Housing: Animals were housed individually in stainless steel cages.
   Diet: Certified Purina Rabbit Chow (#5322), ad libitum
   Water: Filtered tap water, ad libitum
   Environmental conditions:
   Temperature: 67.3±0.4°F.
   Humidity: 64.0±7.8%
   Air changes: Not reported
   Photoperiod: 12 hours

   Acclimation period: 4 weeks

B. STUDY DESIGN:

1. In life dates - start: 7/14/82 end: 7/27/82

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN: 14-Day Dermal Toxicity Study of Gulf Orchard Spray 70 in Rabbits[a]

Test Group	Dose to Animal (g/kg/day)	Male	Female
Vehicle Control (Corn Oil)	0.0	3	3
Low (LDT) (43.1% w/v in corn oil)	1.0	3	3
High (HDT) (Undiluted test substance)	2.0	3 (3)[b]	3 (3)[b]

[a]Data taken from page 10 of this submission (MRID 41368806).
[b]These additional animals were examined and sacrificed after a two-week recovery period during which no treatment with Gulf Orchard Spray 70 was received.

Hair was clipped (electric clippers) from the backs of the test animals prior to study initiation. Animals were fitted with a neck collar to prevent ingestion of the test substance; this collar remained on each animal until sacrifice. Test solutions were applied topically over approximately 10% of the body surface. Following application, the test sites were occluded with a polymer film-covered gauze patch. Trunks of the animals were wrapped with an elastic bandage. At six hours post-dosing, the treatment sites were exposed and excess test substance wiped from the skin. Doses were calculated based on individual body weights and adjusted weekly. Volumes of substances applied varied from 2.0 to 2.32 mL. Treatments were conducted five days per week for a total of two weeks. High-dose recovery animals were examined after a two-week recovery period, during which no treatments were administered.

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3. Preparation and analysis of dosing solutions

Dilutions of test substance were prepared weekly. The corn oil vehicle and high-dose test substance (undiluted Gulf Orchard Spray 70) were administered neat. No data are provided on the concentration, homogeneity, or purity of the test substance, its dilutions, or the vehicle.

Results - No data were submitted.

4. Statistics - Organ weight data were analyzed by mean and standard deviation, Barlett's test, and analysis of variance. If Barlett's test indicated homogeneous data, then Dunnett's test was conducted. If Bartlett's test indicated non-homogeneous data, then a modified t-test was employed. Incidence of microscopic findings were examined using the Kolmogorov-Smirnov two-tailed test.

C. METHODS:

1. Observations:

Animals were inspected twice daily on dosing days and once daily on non-dosing days for mortality and moribundity. Clinical signs were noted once daily on dosing days. Dermal reactions were scored (using a Draize method) twice daily on dosing days: once before dose application and once after removal of the occluding materials at 6 hours of exposure.

2. Body weight

Main-study animals were weighed prior to study initiation, weekly thereafter, and at study termination. Recovery group animals were additionally weighed at the end of the two-week recovery period.

3. Food consumption

Food consumption for each animal was determined daily on each dosing day.

4. Ophthalmoscopic examination

Examinations were not conducted.

5. Blood was collected via the auricular vessels for hematology and clinical analysis from all animals (fasted overnight) prior to study initiation and at main study termination (for recovery animals, blood was additionally collected at sacrifice after two weeks of no treatment). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		Red cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
			Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase	X	Albumin/globulin ratio
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was not collected or analyzed.

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals that died (none) and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination and examined for all animals. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*	XX	Brain*
	Salivary glands*	XX	Heart*		Periph. nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)[T]
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*		Spleen*		Eyes (optic n.)[T]
	Jejunum*		Thymus*		
	Ileum*				
	Cecum*		UROGENITAL		GLANDULAR
	Colon*	XX	Kidneys*+		Adrenal gland*
	Rectum*		Urinary bladder*		Lacrimal gland[T]
XX	Liver*[+]	XX	Testes*[+]		Mammary gland[T]
	Gall bladder*		Epididymides		Parathyroids*[++]
	Pancreas*		Prostate		Thyroids*[++]
			Seminal vesicle		
	RESPIRATORY	XX	Ovaries		OTHER
	Trachea*		Uterus*		Bone
X	Lung*				Skeletal muscle
	Nose			X	Skin
	Pharynx				All gross lesions and masses*
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
[+] Organ weight required in subchronic and chronic studies.
[++] Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations :

1. Toxicity - With the exception of dermal reactions, the incidences of clinical signs in treated animals were similar to those observed in appropriate controls. In the 1 g/kg/day group, sporadic and transient slight erythema and edema were noted, and the incidence was significantly elevated with respect to controls. In the 2 g/kg/day group, all animals exhibited very slight to severe erythema and very slight to moderate edema at some point during treatment. In addition, desquamation occurred in 4/12 animals in this group, and animals in this treatment group continued to exhibit desquamation after the two-week recovery period. Animals in the corn oil control group exhibited only sporadic very slight erythema and edema.

2. Mortality - There were no unscheduled deaths; all animals survived to study termination. No moribund animals were observed.

B. Body weight: As shown in Table 2, there were no biologically significant treatment-related effects on body weight.

Table 2. Body Weights (Grams) of Animals Treated Dermally for 14 Days with Gulf Orchard Spray 70[a]

Dose (g/kg/day)	Sex	Study Day -1	Study Day 7	Study Day 14	Study Day 28
0 (Corn Oil Control)	Male	2851.0± 171.6[b] (100)[c]	2749.3± 156.2 (100)	2935.3± 190.3 (100)	--
	Female	2833.7± 177.4 (100)	2775.7± 175.4 (100)	2918.0± 209.9 (100)	--
1.0 (Low Dose) (LDT)	Male	2756.7± 142.2 (97)	2575.3± 92.6 (94)	2648.7± 23.2 (90)	--
	Female	2815.7± 222.3 (99)	2783.0± 271.6 (100)	2909.7± 233.4 (100)	--
2.0 (High Dose) (HDT)	Male	2763.3± 54.0 (97)	2722.0± 47.4 (99)	2680.0± 164.8 (91)	--
	Female	2798.3± 82.5 (99)	2681.7± 342.7 (97)	2751.7± 460.4 (94)	--
2.0 (After 2-Week Recovery)	Male	2476.3± 109.9 (100)[d]	2335.7± 295.5 (94)	2351.0± 156.7 (95)	2322.7± 197.7 (94)
	Female	2669.0± 100.7 (100)	2626.7± 15.1 (98)	2666.0± 144.2 (100)	2877.0± 160.4 (108)

[a]Data extracted from pages 27-30 of this submission (MRID 41368806).
[b]Mean ± standard deviation from the mean.
[c]Figures in parentheses indicate percentages of appropriate control values.
[d]For recovery animals, the day -1 body weight has been set as 100% (internal control value).

C. Food consumption

1. Food consumption - There were no biologically significant treatment-related effects on food consumption.

2. Food efficiency - Food efficiency was not determined.

D. Ophthalmoscopic examination - Ocular examinations were not conducted.

E. Blood work:

1. Hematology - The hematological values observed for animals treated with Gulf Orchard Spray 70, at both dose levels tested, were comparable to those obtained for the corn oil control animals.

2. Clinical Chemistry - The clinical chemistry values obtained for animals treated with Gulf Orchard Spray 70 at both dose levels were comparable to those exhibited by animals in the corn oil control group.

F. Urinalysis - No urinalyses were conducted.

G. Sacrifice and Pathology:

1. Organ weight - Organ weights and organ/body weight and organ/brain weight ratios were comparable to control values for the high-dose (2 g/kg/day) group animals after two weeks of recovery. This was also true of animals in both treated groups (1.0 g/kg/day and 2.0 g/kg/day) that were sacrificed after 14 days on test, with the following exception: although the absolute weights of the kidneys were not statistically significantly different from control values, the kidney/body weight ratios were increased for both males and females in both treated groups. However, the kidney/brain weight ratios were not correspondingly increased for these groups, and no gross or microscopic changes in these kidneys were noted at necropsy. Therefore, the biological significance of these increased kidney/body weight ratios is unknown. Representative organ weight and organ to body weight ratio data are presented in the following Table 3:

Table 3. Representative Organ and Organ/Body Weight and Organ/Brain Weight Ratio Data for Rabbits Dermally Exposed for 14 Days to Gulf Orchard Spray 70[a]

Dose Level (g/kg/day)	Body Weight (g)	Brain Weight (g)	Weight of Organ (g)	Organ to Body Weight Ratio	Organ to Brain Weight Ratio
Females: Left Kidney					
0	2918.0± 209.9[b]	9.3593± 0.4086	8.5053± 0.9111	0.2910± 0.0112	0.9118± 0.1250
1.0	2909.7± 233.4	9.7470± 0.1831	8.4937± 0.8300	0.2917± 0.0049	0.8710± 0.0768

2.0	2877.0± 160.4	9.3133± 1.0561	9.1800± 1.6362	0.3333± 0.0044**	0.9891± 0.1578
Males: Left Kidney					
0	2935.3± 190.3	9.3867± 0.2373	8.8020± 0.6701	0.3001± 0.0168	0.9380± 0.0732
1.0	2648.7± 23.2	9.4697± 0.7799	9.0567± 0.3729	0.3418± 0.0113*	0.9587± 0.0460
2.0	2680.0± 164.8	9.2393± 0.1407	9.0747± 0.1146	0.3395± 0.0216	0.9825± 0.0271
Females: Right Kidney					
0	2918.0± 209.9	9.3593± 0.4086	8.3340± 0.7262	0.2854± 0.0076	0.8933± 0.1091
1.0	2909.7± 233.4	9.7470± 0.1831	8.2887± 1.1057	0.2842± 0.0168	0.8502± 0.1086
2.0	2877.0± 160.4	9.3133± 1.0561	8.6503± 1.6015	0.3138± 0.0067*	0.9315± 0.1501
Males: Right Kidney					
0	2935.3± 190.3	9.3867± 0.2373	8.3793± 0.4062	0.2858± 0.0086	0.8927± 0.0389
1.0	2648.7± 23.2	9.4697± 0.7799	8.6777± 0.4115	0.3276± 0.0157**	0.9189± 0.0563
2.0	2680.0± 164.8	9.2393± 0.1407	9.0933± 0.4472	0.3395± 0.0043**	0.9843± 0.0499

[a]Data extracted from pages 27-29, 54, 59, and 62 of this submission (MRID 41368806).
[b]Represents the mean ± standard deviation from the mean.
*Indicates that $p < 0.05$.
**Indicates that $p < 0.01$.

2. Gross pathology - No treatment-related lesions were observed at gross necropsy. Two control animals (one male and one female) exhibited focal areas of thickening of the skin. One control male in the recovery group exhibited epidermal erosion. In the recovery group, two high-dose (2.0 g/kg/day) males and one high-dose female exhibited abnormal liver color; however, no microscopic abnormalities were found in these livers and the biological significance of these gross observations are therefore unknown.

3. Microscopic pathology -

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a) Non-neoplastic - The only treatment-related microscopic lesions observed consisted of epidermal acanthosis and hyperkeratosis of the skin at the treatment site in high-dose (2.0 g/kg/day) males and females. Thickening of the epidermis occurred, due to hyperplasia of the basilar epithelium and keratin accumulation on the skin surface. Treatment-unrelated lesions were consistent with an infection by the protozoan parasite, Encephalitozoon cuniculi, or were those normally observed as a result of common diseases in the New Zealand White rabbit.

b) Neoplastic - No treatment-related neoplastic lesions were detected.

III. DISCUSSION

A. The only biologically significant effects observed following the dermal administration of Gulf Orchard Spray 70 to New Zealand White rabbits were histologically confirmed skin lesions at the treatment site. Although the testing facility noted that body weights were generally decreased in treated animals, these decreases represented 90-100% of control values at 14-day sacrifice and, therefore, are not considered to be biologically significant. This study has been classified unacceptable, but upgradable; however, based on the lack of any systemic (non-dermal) effects at dose levels up to 2.0 g/kg/day, requiring a repetition of this study would not yield any meaningful additional toxicological information for this substance.

B. Study deficiencies - No data were submitted on the purity or stability of the test substance. Dosing was conducted 5 days/week for a total of 2 weeks rather than the guideline procedure of at least 5 days/week for a total of 3 weeks. The following clinical chemistry blood parameters were not determined: chloride, phosphorus, and SGOT. In addition, no blood clotting measurement was determined during hematology analyses.