UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 9 1987

005679

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA File Symbol [redacted] 53871-E
Stirrup-M Pheromone for use as a Mite Attractant in [redacted]
Pesticide Formulations (1.7% a.i.)

FROM: William S. Woodrow, Ph.D.
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Willie Nelson, PM Team 17
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

CAS # 801
ACC # 264

Registrant: Fermone Chemicals
305 South Second Avenue, Suite 101
Phoenix, AZ 85003

Action Requested: [redacted]

In a letter from Andrew Jovanovich of the Technology Services Group, representing Fermone Chemicals, Inc., dated August 20, 1986, the following requests to the Agency were made on behalf of the product, Stirrup-M:

1. A registration for Stirrup-M as a pesticide product to be used in conjunction with other registered miticides;

2. A general exemption from tolerance for the active ingredients in Stirrup-M;

3. A clearance for use of Stirrup-M as an inert ingredient in registered miticides; and

1/84

005679

4. A special exemption from tolerance for use of Stirrup-M as an inert additive in registered miticides.

Additional toxicity data were submitted in support of Stirrup-M.

Background Information:

A previous request for registration of Stirrup-M stated that the Stirrup-M active ingredient is known as Farnesol, which has the following chemical name:

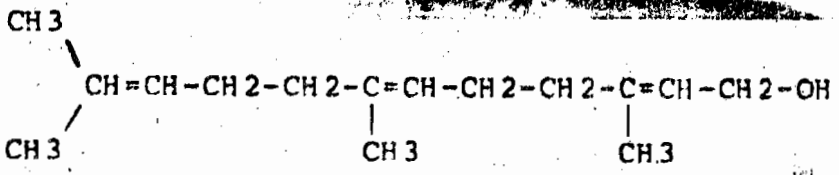
3,7,11-Trimethy-2,6,10-dodecatriene-1-ol.

(See Woodrow's memorandum of July 9, 1986 to Willie Nelson.)

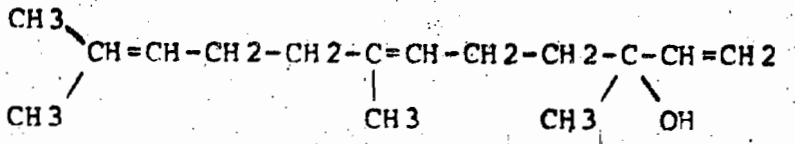
Some acute toxicity studies were submitted with the previous Stirrup-M correspondence, and were evaluated in Woodrow's July 9, 1986 memorandum.

The present reply to the August 20, 1986 Jovanovitch letter states that Stirrup-M is actually composed of two terpene alcohols named FARNESOL and NEROLIDOL, whose chemical formulations are presented below:

FARNESOL -



NEROLIDOL -



Acute toxicity studies conducted separately with Farnesol and with Nerolidol were submitted with the August 20, 1986 Jovanovitch letter, in support of Stirrup-M registration, as well as a G-P sensitization study conducted with the Stirrup-M.

Recommendations:

1. The use of Stirrup-M mixed with miticide pesticides to control mites on food crops is not supported toxicologically.

Reply to specific requests reiterated under Action Requested in the present memorandum:

i. (Request) A registration for Stirrup-M as a pesticide product to be used in conjunction with other registered miticides.

Reply - Stirrup-M is a pheromone product intended for crop use, and as such, all of the toxicity data requirements under Tier 1 for Biochemical Pesticides must be submitted and accepted.

The following requested toxicity data under Tier 1 tests for Biochemicals remain data gaps:

- o One 90-day subchronic feeding study
- o One teratology study
- o A battery of mutagenicity studies to detect:
  - Gene mutation
  - Structural chromosomal aberrations
  - Other genotoxic effects, e.g., numerical [redacted] direct DNA damage and repair.
- o The surfactant [redacted] contained in the product formulation must be cleared by the Agency for food use.

(See Woodrow's memorandum of July 9, 1986 for reference to current data gaps and the need for [redacted] clearance.)

ii. (Request) A general exemption from tolerance for the active ingredients in Stirrup-M.

Reply - An exemption from tolerance for the Stirrup-M active ingredients may be considered, upon submission of all of the outstanding data (cited under i. above, in acceptable form), and upon proper verification of food use clearance for [redacted]

iii. (Request) A clearance for use of Stirrup-M as an inert ingredient in registered pesticides.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Reply - Stirrup-M cannot be cleared as an inert ingredient for use in registered pesticides.

The Federal Insecticide, Fungicide, and Rodenticide Act, as amended (PL 92-516 October 21, 1972 as amended by PL 94-140 November 28, 1975 and PL 95-396 September 30, 1978, under "Section 2. Definitions" "(a) Active Ingredient - the term 'active ingredient' means (1) in the case of a pesticide other than a plant regulator, defoliant, or desiccant, an ingredient which will prevent, destroy, repel, or mitigate any pest."

Stirrup-M is intended to mitigate certain mite species (Tetranychid species) by causing behavior modification.

iv. (Request) A special exemption from tolerance for use of Stirrup-M as an inert additive in registered miticides.

Reply - The special nature of Stirrup-M has already been considered in that this product was designated a biochemical pesticide, for which only Tier 1 biochemical pesticide data are required.

2. Previously submitted toxicity data:

a. Acute Oral LC_{50} - rat, using Stirrup-M (0.923% ai - Multi-methyl alkenol).

A O LD_{50} > 5050 mg/kg.

Toxicity Category: IV

Classification: Core Guideline Data.

b. Acute Dermal LD_{50} - rabbit, using Stirrup-M (0.923% Multi-methyl alkenol).

A D LD_{50} > 2020 mg/kg.

Toxicity Category: III

Classification: Core Guideline Data.

c. Acute Inhalation LC_{50} - rat, using Stirrup-M (0.923% Multi-methyl alkenol).

A I LC_{50} > 3.37 mg/L for t = 4 hours.

Toxicity Category: III

Classification: Core Minimum Data.

d. Primary Ocular Irritation - rabbit, using Stirrup-M (0.923% Multi-methyl alkenol).

The test material produced mild ocular irritation in washed and unwashed rabbit eyes.

Toxicity Category: III

Classification: Core Guideline Data.

e. Primary Dermal Irritation - rabbit, using Stirrup-M (0.923% Multi-methyl alkenol).

The test material caused slight irritation at 1 hour, which cleared within 24 hours.

Toxicity Category: IV

Classification: Core Guideline Data.

3. Data reviewed in the present submission:

a. Acute Oral Toxicity - rat, using Farnesol (purity-composition not given).

Acute Oral LD_{50} - Farnesol (tentatively) > 5050 mg/kg (5.6 mL/kg).

Toxicity Category: IV

Classification: Tentatively Core Minimum Data (% purity and composition of test material must be provided).

b. Acute Oral Toxicity - rat, using Nerolidol (purity-composition not given).

Acute Oral LD_{50} - Nerolidol (tentatively) = 5486 mg/kg.

005679

Toxicity Category: IV

Classification: Tentatively Core Minimum Data (% purity and composition must be stated).

c. Acute Dermal Toxicity - rat, using Farnesol, (purity and composition not given).

Acute Dermal LD_{50} - Farnesol, (tentatively) > 2010 mg/kg (2.23 mL/kg (intact skin only)).

Toxicity Category: III

Classification: Tentatively Core Minimum Data (% purity and composition must be stated).

d. Acute Dermal LD_{50} - Nerolidol, using the rabbit (% purity and composition not given).

Acute Dermal LD_{50} - Nerolidol (tentatively - intact skin only) > 2010 mg/kg (2.27 mL/kg).

Toxicity Category: III

Classification: Tentatively Core Minimum Data (% purity and composition must be stated).

e. Rabbit Eye Irritation, using Farnesol (% purity and [redacted]

P.I. score = 6.7/110. Farnesol was minimally irritating.

Toxicity Category: III

Classification: Tentatively Core Minimum Data (% purity and composition must be stated).

f. Rabbit Eye Irritation, using Nerolidol (% purity and composition not stated).

P.I. score = 8.0/110. Nerolidol was minimally irritating.

Toxicity Category: III

Classification: Tentatively Core Minimum Data (% purity and composition of the test material must be stated).

5' Guinea Pig Sensitization using Stirrup M (EPA File # 53871-E). Purity and composition not stated. Not a sensitizer. 6
Classification: Tentative Core Guideline pending

005679

g. Rabbit Skin Irritation, using Farnesol (% purity and composition not given).

P.I. score = 0.2, a practically nonirritating material.

Toxicity Category: IV

Classification: Tentatively Core Minimum Data (the % purity and composition of the test material must be stated).

h. Rabbit Skin Irritation, using Nerolidol (% purity and composition not given).

P.I. score = 1.2/8, Nerolidol is a slight dermal irritant.

Toxicity Category: III

Classification: Tentatively Core Minimum Data (the % purity and composition of the test material must be given).

i. Rat Acute Inhalation Toxicity, using Farnesol (% purity - composition not given).

Rat LC_{50} ([redacted]

Males = 0.828
Females = 1.02
Overall = 0.917

Toxicity Category: II

Classification: Tentatively Core Guideline Data (% purity and composition of the test material must be stated).

j. Rat Acute Inhalation Toxicity, using Nerolidol (% purity and composition not stated).

Male and female rat LC_{50}, using Nerolidol = [redacted] mg/L (95% C.L. = [redacted] to [redacted] mg/L).
1.46 0.826 2.53

Toxicity Category: II

Classification: Tentatively Core Guideline Data (the % purity and test material composition must be stated).

k. Ames mutagenicity study, using a number of test chemicals, including Farnesol.

Classification: Not acceptable. (The tester did not state the number of replicate plates per test. Positive and negative control test results were not presented, and proof that the Farnesol test concentration employed approached Salmonella typhimurium lethal cell tolerance(s) was not presented.) Raw data was also not submitted.

4. The percent purity and complete chemical composition of the Farnesol and Nerolidol test materials used in the toxicity tests listed under 3., above (reviewed in the present submission) must be provided to the Agency.

5. The product label precautionary signal word and statement are satisfactory.

6. Review of data contained in the present submission follows (see Data Evaluation Records).

7. The previous review of this product indicated only c. 923% ai in the form of Farnesol with 99.077% inert ingredients. This [redacted] indicates 1.76% ai and the remainder inert ingredients. It is pointed out here that the change in the percent of actives does not mean a change in the composition of the formulation but rather the correct declaration of active ingredient (ie. one active was incorrectly considered an inert).

DATA EVALUATION RECORD 005679

Subject: Guinea Pig Skin Sensitization

Test Material: Stirrup-M. Viscous, off-white liquid. Purity and composition not given.

Positive Control Material. 1-chloro-2,4, dinitrobenzene; LOT A18, 0.05% w/v in ethanol.

EPA File Symbol: 53871-E

Accession No: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Project No: 4033-86

Report Date: April 11, 1986

Project Director: Joseph L. Maedgen

Toxicity Category: Nonsensitizer

Classification:

Core-Guideline Data [redacted] submission composition and purity of test material.

Materials and Methods:

Twenty albino short-haired male Hartley guinea pigs were divided into a positive control of 10 animals (Group I) and 10 animals treated with Stirrup-M (Group II), to determine the skin sensitizing potential of Stirrup-M.

One-half mL of a 0.05% w/v solution of 2,4-dinitrochlorobenzene was introduced beneath a 1.5 inch x 2 inch gauze patch secured to a 1.5 inch x 2 inch piece of adhesive on clipped and depilated left dorsal sites on each of 10 positive control guinea pigs. The same procedure for the positive control guinea pigs was conducted (using the same application sites) in days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, and 36. Following each treatment, the entire trunk of each animal was wrapped with 4 mil clear polyethylene film to secure the patches. Each treated animal was then placed in a restrainer for approximately 6 hours exposure to the positive control chemical. At the end of the exposure period, wrappings and patches were removed.

Observations for skin reactions were made approximately 24 hours after treatment for each test site. Also, observations for skin reactions were made after 48 hours for treatments 1 and 10, and after the final challenge treatment on day 36.

On the final treatment day (day 36) all animals were treated with positive control material in a similar manner, using 0.5 mL of the positive control chemical; however, application was made to a new skin site on the left back dorsal area.

The ten male animals tested with Stirrup-M were treated in exactly the same manner as the positive control animals, except that undiluted Stirrup-M test material was used. The skin reaction scoring scale is presented below:

Quoted from the tester's report

SKIN REACTION GRADING SCALE

Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar [redacted]	4

Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

End of quotation

An average score for each time period was attained by adding all the scores for each time period and dividing by the number of test sites scored for that time period.

A positive sensitization reaction observed on the final (challenge) application day would consist of a marked increase above the reactions observed after the initial treatment (sensitizing application).

Results:

The test group (Stirrup-M) average test scores were 0.0 for the initial treatment, 0.0 for the final original test site treatment (treatment 10), and 0.0 for the different (virgin) challenge test site.

The average skin reaction scores for the positive control group of 10 guinea pigs (2,4-dinitrochlorobenzene) were: 0.0 for the initial scoring, 2.5 for the different (virgin) challenge test site, and 3.4 for the final (No. 10) sensitization test site.

Conclusions:

1. The 2-4, dinitrochlorobenzene positive control animal demonstrated a marked (positive) sensitization skin reaction.

2. Stirrup-M, tested undiluted on male Hartley strain guinea pigs, did not produce a skin senisitizing reaction.

Classification:

Core-Guideline Data (tentative) pending submission of composition and purity of test material.

89847:Kocialski:HED-01:KENCO:12/19/86:3/2/87:DJ:VO
F:88985:Kocialski:HED-01:KENCO:12/30/86:2/7/87:eg:vo

DATA EVALUATION RECORD

005679

Subject: Acute Oral Toxicity (LD_{50}) - Rat

Test Material: Farnesol (terpene alcohol)
Density 0.902 g/mL
Purity not stated

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4028-86, May 7, 1986

Author: Project Director, Joseph L. Maedgen, M.S.

Toxicity Category: IV

Classification: Tentatively Core Minimum Data

Materials and Methods:

Five male and five female Sprague-Dawley rats were each administered single doses of 5050 mg/kg (5.60 mL/kg) undiluted Farnesol by intubation.

Observations for mortality and toxic effects were made 3X on the day of treatment and at least once daily for 14 days. Body weights were recorded pre- and postdosing on days 7 and 14. Gross necropsy examinations were conducted on each animal at study termination.

Results:

No mortality, all animals gained weight during the study. A variety of toxic symptoms were observed in both males and females prior to experiment termination, such as: piloerection constricted or dilated pupils, diarrhea, polyuria, ptosis, and activity decrease. At necropsy, no observable findings were noted for any animals.

Conclusions:

The acute oral LD_{50} > 5050 mg/kg (5.60 mL/kg) for both sexes. Calculated by the method of Litchfield and Wilcoxon (using Farnesol).

Toxicity Category: IV

Classification:

Tentatively Core Minimum Data (the [redacted] must provide the percent purity and composition of the test material).

E: Piloerection was observed in all males and females for 5 days. Severity of effect was reported from very slight to severe. Ptosis was observed in the majority of males early and demonstrated no time trend with regard to the number responding and the severity of effect. Ptosis was observed in [redacted] females [redacted] days 1-3. Polyuria was observed in both sexes of females and appeared to follow a "bell-shaped" curve with the fewest number of males (5) and females (5) being affected on days 2 and 1 respectively. Restricted guards were observed in both sexes with males responding earlier. All signs were absent the day (See attachment)

Table 2

RAT ACUTE ORAL TOXICITY

Pharmacologic and/or Toxicologic Effects

Test Material: FARNESOL

Dose Level: 5050 mg/kg (5.60 ml/kg)

Reaction and Severity	Time After Treatment: HOURS ½	3	6	DAYS 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Piloerection (v-e)	4	5	5	5	5	5	5	5	1	0	0	0	0	0	0	0	0
Constricted Pupils (s-e)	4	5	5	5	2	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Activity Decrease (v-s)	3	3	4	4	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Ptosis (s-m)	4	5	4	5	3	[illegible]	2	1	0	0	0	0	0	0	0	0	0
Mucoid Diarrhea (v-m)	2	4	1	0	1	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Respiratory Gurgle (s)	1	0	0	0	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Polyuria (v-m)	0	1	1	3	5	[illegible]	2	0	0	0	0	0	0	0	0	0	0
Females																	
Piloerection (v-e)	5	5	5	5	5	[illegible]	5	2	1	0	0	0	0	0	0	0	0
Exophthalmos (s-m)	1	1	1	1	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Dilated Pupils (s)	3	5	2	0	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Diarrhea (v-e)	1	5	5	5	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Activity Decrease (v-s)	0	3	3	3	2	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Polyuria (v-e)	0	4	2	5	3	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Sensitive to Touch (s-m)	0	1	1	1	1	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Constricted Pupils (s-m)	0	0	1	3	2	[illegible]	1	1	0	0	0	0	0	0	0	0	0
Ptosis (s)	0	0	0	3	2	[illegible]	0	0	0	0	0	0	0	0	0	0	0
Lacrimation (s)	0	0	0	1	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0

Note: Numbers indicate surviving animals exhibiting reaction.
Time of death indicates time of discovery after death (if discovery was between scheduled observations, death is presented at next observation time).

v - very slight; s - slight; m - moderate; e - extreme.

Acute Dermal

Skin Irritation

Eye Irritation

005679

DATA EVALUATION RECORD

Subject: Acute Oral Toxicity - Rat (LD_{50})

Test Material: NEROLIDOL (terpene alcohol)
Density 0.887 g/mL
Purity not stated

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Oak Road
Houston, TX 77055

Report No./Date: Project No. 4023-86, April 16, 1986

Author: Project Director, Joseph L. Maedgen, M.S.

Toxicity Category: IV

Classification: Tentatively Core Minimum Data

Materials and Methods:

Five male and fifteen female Sprague-Dawley rats were dosed by intubation as follows:

Quoted from the tester's report:

Dose (mg/kg)	Male	Female
4000		5
5050	5	5
6300		5

End of quotation

Test animals were administered undiluted test material. Observations for mortality and toxic signs were made 3X on the day of treatment and at least once per day for 14 days. Gross necropsies were conducted on each animal dying, or at experiment termination (14 days).

Results:

Mortality occurred as follows:

Quoted from tester's report:

Dose (mg/kg)	Male	Female	Combined Mortality
4000		0/5	0/5
5050	0/5	3/5	3/10
6300		3/5	3/5

End of quotation

A number of toxic signs were observed, such as: activity decrease, ataxia, constricted pupils, dilated pupils, diarrhea, lacrimation, nasal discharge, piloerection, ptosis, and salivation. (See also attached)

No gross necropsy findings were evident at the 4000 mg/kg dose level.

None of the male rats and two female rats at the 5050 mg/kg dose level exhibited gross necropsy findings; however, the three females that died showed signs of chromodacryorrhea, epistaxis, polyuria, and discolored fluid in small intestines.

The surviving two females dosed at 6300 mg/mL showed no gross necropsy findings. The remaining three females that died at this dose level exhibited such findings as: salivation, polyuria, and dark brown liquid in the intestinal tract.

Conclusions: for females

The acute rat oral LD_{50} using Nerolidol, calculated by the method of Litchfield and Wilcoxon was 5486 mg/kg (95% CL = 4445 to 6773 mg/kg). The acute oral LD_{50} for males was > 5050 mg/kg.

Toxicity Category: IV

Classfication:

Tentatively Core Minimum Data (the percent purity and composition of the test material must be stated).

[redacted]

Signs observed for the respective sexes at the noted doses were as follows:

1. 4000 mg/kg females: Slight to extreme piloerection was observed in females for as long as 10 days with as many as 4/5 females responding through day 7. The effect was reported as slight to extreme. Polyuria was observed in 4/5 animals through day 4 and was reported as very slight to extreme.

2. 5050 mg/kg females: Piloerection was observed for as long as 12 days with at least 2/5 animals responding as late as 11 days. Severity was rated as slight to extreme. Polyuria was observed for as long as 5 days with as many as 4/5 animals responding through day 3. Severity was rated as very slight to extreme. Constricted pupils were observed for as long as 10 days with as many as 3/5 animals responding through day 4. The effect was rated slight to moderate. Ptosis was also observed from days 1 to 5 in as many as 3/5 animals. Severity was rated as slight to extreme.

3. 6300 mg/kg females: Slight to extreme piloerection was observed through 14 days with at least 2/5 animals responding through day 8. Exopthalmos was also observed through day 7 with at least 2/5 animals responding through day 6. The effect was rated slight to moderate. Ataxia was also observed in 4/5 animals for as long as 4 days and was rated as very slight to extreme. Slight to extreme polyuria was also observed in 4/5 animals through day 4.

4. 5050 mg/kg males: Slight to extreme piloerection was observed in as many as 5/5 males for 8 days. Very slight to extreme polyuria was observed in 4/5 animals for at least 2 days. Slight to moderate activity decrease was observed for 5/5 males at day 1.

Body weight gain was noted in all survivors whereas those animals that died on experiment lost weight.

005679

DATA EVALUATION RECORD

Subject: Acute Dermal Toxicity, Rat (LD_{50})

Test Material: Farnesol (Terpene alcohol) Density 0.902 g/mL
Purity not stated

EPA File Symbol: [redacted] 53571-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4029-86, April 9, 1986

Author: Project Director, Joseph L. Maedgen, M.S.

Toxicity Category: III

Classification: Tentatively Core Minimum Data

Materials and Methods:

Five male and five female NZW rabbits were clipped free of hair on the dorsal trunk surface to expose not less than 10 percent of the total body surface on the day prior to treatment.

Surgical gauze was applied to animal trunks at treatment sites and held in place with tape. Entire trunks were then wrapped with semipermeable material to retard evaporation.

All animals were treated with 2010 mg/kg (2.23 mL/kg) of undiluted test material by inserting a syringe under the wrappings and gauze and spreading it evenly over the exposure area, to intact skin (no dermal areas were abraded). The tape was then resealed.

Twenty-four hours following treatment, all wrappings were removed, and the exposed areas gently washed. Observations for toxic effects were made at 1/2, 3, and 6 hours, and at least once daily for 14 days posttreatment. Body weights were measured on days 0, 7, and 14 or at time of death. A gross necropsy was conducted on animals dying during the study, or at experiment termination (14 days).

Results:

One female rabbit died on day 3. One male and another (other than the one that died) female rabbit lost weight during the observation period, while the remaining rabbits gained weight.

Three male rabbits and two female rabbits did not exhibit necropsy findings. Two males and three female rabbits (including the female that died) exhibited necropsy findings, such as: signs of diarrhea and emaciation, discolored liquid in large intestine and ceceum, discolored livers, gas in gastrointestinal tracts.

Conclusions:

The acute dermal LD_{50} using Farnesol in rabbits was > 2010 mg/kg (2.23 mL/kg) - intact skin only.

Toxicity Category: III

Classification:

Tentatively Core Minimum Data (the % purity and composition must be stated).

005679

DATA EVALUATION RECORD

Subject: Acute Dermal LD_{50} - Rabbit

Test Material: Nerolidol. Density 0.887 g/mL. Purity and composition not given.

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4024-86, April 9, 1986

Author: Project Director, Joseph L. Maedgen, M.S.

Toxicity Category: III

Classification: Tentatively Core Minimum Data

Materials and Methods:

The hair was clipped from the dorsal surface of the trunk of five male and five female NZW rabbits, prior to the day of testing. Two layers of surgical gauze was applied to the trunk of each animal and held in place with tape. The entire trunk of each animal was then wrapped with semipermeable material to the retard test material evaporation.

The test material was introduced under the wrappings using a syringe needle to deliver 2010 mg/kg (2.27 mL/kg) per each animal of undiluted Nerolidol, which was spread over the prepared skin surface using the same needle. The wrappings were then resealed and left in place for 24 hours, at which time all wrappings were removed, and treated sites were washed with water.

Observations for toxic signs, mortality, and skin irritation were made at 1/2, 3, and 6 hours, and daily thereafter for 14 days. Individual body weights were measured on days 0, 7, and 14. A gross necropsy examination on each animal was made at termination (14 days).

Results:

No mortality. All animals gained weight during the 14-day observation period. Only one animal exhibited findings at necropsy; a female showed signs of diarrhea, and gastrointestinal tract distended with gas.

Conclusions:

The acute dermal LD_{50} using Nerolidol, in rabbits (intact skin) is > 2010 mg/kg (2.27 mL/kg).

Toxicity Category: III

Classification:

Tentatively Core Minimum Data (the % purity and chemical composition of the test material must be stated).

005679

DATA EVALUATION RECORD

Subject: Rabbit Eye Irritation Study

Test Material: Farnesol. Clear, pale yellow liquid. Purity not stated.

EPA File Symbol: [redacted] 5383-71-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4030-86, March 20, 1986

Author: Project Leader, Joseph L. Maedgen

Toxicity Category: III

Classification: Tentatively Core Minimum Data

Materials and Methods:

One tenth mL of undiluted test material was instilled into the right eye of each of nine NZW rabbits by pulling the lower lids away from the eyeball. Eyelids were then gently held together for 1 second. Three of the treated eyes were then flushed with water for 1 minute beginning 30 seconds after treatment; left (untreated) eyes or the nine test rabbits served as controls.

The eyes of all animals were examined and scored for irritation at 1, 24, 48, and 72 hours, and at 4 days post-treatment. The corneas of all treated eyes were reexamined immediately after the 24-hour examination with 0.2% fluorescein sodium ophthalmic solution. An irritation score was determined, apparently according to the method of Draize (total possible score of 110).

Results:

Primary irritation score for unwashed eyes was 6.7/110 (minimally irritating). The P.I. score for washed eyes was 5.3 (minimally irritating).

Toxicity Category: III

Classification:

Tentatively Core Minimum Data (the registrant must state the percent purity and composition of the test material employed in order to elevate the test to Core Minimum).

DATA EVALUATION RECORD

005679

Subject: Rabbit Eye Irritation Study

Test Material: Nerolidol. Clear pale yellow liquid. No statement on purity or composition.

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4025-86, March 20, 1986

Author: Project Leader, Joseph L. Maedgen

Toxicity Category: III

Classification: Tentatively Core Minimum Data

Materials and Methods:

One tenth mL of undiluted Farnesol was instilled into the conjunctival sac of the right eye of each of nine NZW rabbits by pulling the lower eyelid away from the eyeball. Eyelids were then gently held together for 1 second. Three of the nine treated eyes were washed with water for 1 minute beginning 30 seconds after treatment. Untreated left eyes served as controls.

The treated eyes of all animals were examined and scored for irritation at 1, 24, 48, and 72 hours and at 4 days after treatment. The corneas of all treated eyes were reexamined immediately after the 24-hour observation with 0.2% fluorescein sodium ophthalmic solution.

An irritation score was determined for each animal at each observation time for unwashed and washed eyes, according (apparently) to the Draize system.

Results:

A maximum average irritation score of 8.0/110 was determined for nonwashed eyes (minimally irritating). The maximum irritation score for washed eyes was 7.3 (minimally irritating). Conjunctival irritation was absent (cleared) by day 7.

Toxicity Category: III

Classification:

Tentatively Core Minimum Data (the purity and composition of the test material used must be provided).

005679

DATA EVALUATION RECORD

Subject: Rabbit Skin Irritation

Test Material: Farnesol. Clear pale yellow liquid. Percent purity and composition were not given.

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4031-86, March 20, 1986

Author: Project Leader, Joseph L. Meadgen

Toxicity Category: IV

Classification: Tentatively Core Minimum Data

Materials and Methods:

One half mL of undiluted test material was placed under surgical gauze patches two layers thick secured at previously clipped skin sites on the anterior right quadrant of the dorsal trunk area of each of six NZW rabbits. Each patch covering treated skin sites were then further protected by wrapping the animals' entire trunk with a semipermeable dressing.

Four hours following test applications, all wrappings were removed, and the test sites were washed with water. Treated animals were observed for erythema, eschar, and edema formation at 1, 24, 48, and 72 hours after washing.

Irritation scores were compiled, apparently according to the method of Draize, with a maximum possible score of 8.0 (all of the erythema and edema scores were added and the result divided by the number of animals tested).

Results:

One rabbit (only) gave a response at the 1-hour scoring period. The calculated P.I. score was 0.2, which indicates a practically nonirritating material.

Conclusion:

The Primary Irritation dermal score for Farnesol was determined to be 0.2, which indicates a practically nonirritating material.

Toxicity Category: IV

Classification:

Tentatively Core Minimum Data (the test material purity and composition must be submitted).

005679

DATA EVALUATION RECORD

Subject: Rabbit Skin Irritation

Test Material: Nerolidol. Clear, pale yellow liquid. Purity and composition of test substance was not provided.

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4026-86, March 28, 1986

Author: Project Leader, Joseph L. Meadgen

Toxicity Category: III

Classification: Tentatively Core Minimum Data

Materials and Methods:

Six NZW rabbits were prepared for skin testing by clipping the right anterior quadrant of the dorsal trunk area free of hair approximately 24 hours prior to test. One half mL of undiluted Nerolidol was placed under a double layer of gauze at the prepared skin test sites on each of the six rabbits. Gauze patches were secured in place with tape. The entire trunks of the test animals were wrapped with semipermeable dressing to prevent loss of the test substance.

Four hours after test application, all wrappings were removed from the rabbits and the treated sites were washed with water. Each animal was scored for erythema and edema, apparently according to the method of Draize, at 1, 24, 48, and 72 hours after washing, and at 4 and 7 days following washing. The erythema and edema scores were added and the sum divided by the number of animals tested to determine the maximum score.

Results:

A maximum irritation score of 1.2 (possible score of 8.0) was determined, which indicated that Nerolidol is a slight skin irritant. Edema was exhibited at each scoring period through day 4.

Conclusions:

The primary dermal irritation score (P.I. score) was 1.2/8, which indicated Nerolidol is a slight dermal irritant.

Toxicity Category: III

Classification:

Tentatively Core Minimum Data (the percent test material purity and composition must be provided).

005679

DATA EVALUATION RECORD

Subject: Rat Acute Inhalation Toxicity

Test Material: Farnesol. Clear, pale yellow liquid. Percent purity and composition were not given.

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4032-86, June 24, 1986

Author: Project Leader, Joseph L. Maedgen

Toxicity Category: II

Classification: Tentatively Core Guideline Data

[redacted]

Materials and Methods:

Twenty male and twenty female Sprague-Dawley rats were divided into four groups of five males and five females each, and separately exposed for 4 hours (high dose) to four different aerosol concentrations of undiluted Farnesol (amount of exposure controlled by exposure time periods).

During the exposures, the animals were housed in a 500 liter stainless steel dynamic flow inhalation chamber.

The aerosol was generated by pumping the test material through a pressure-operated air nozzle, and the concentrated aerosol was then diluted with dry filtered air and was then drawn into the exposure chamber. Air flow into the chamber was regulated by a critical, calibrated orifice.

The aerosol chamber concentration was measured analytically at least once per hour (Tracor model 560 gas chromatograph), and was also measured gravimetrically once per hour (taken from the animal breathing zone and passing a known volume of aerosol through a preweighed filter and dividing the passed volume of air into the filter collected weight). The aerosol was also measured nominally by dividing the total amount of air passed through the exposure chamber into the weight loss exhibited by the test material reservoir at the end of an exposure period.

Treated animals were observed for mortality, pharmacologic/toxic effects frequently on the day of exposure and at least once daily thereafter for 14 days. Body weights were recorded on days 7, 14, and at time of discovery after death. Gross necropsies were conducted at termination, or at time of discovery after death.

Particle size measurements were made using an Anderson cascade impactor.

Results:

Analytical Aerosol Concentrations - Note that different concentrations were achieved by varying the length of exposure time, rather than the varying concentration of test material to be aerosolized.

By gas chromatography:

Average of 4 samples per concentration:

	#1	#2	#3	#4
Mean Concentration (mg/L)	0.389	0.704	0.974	1.54

(Thus, No. 1 group of animals breathed an aerosol concentration of 0.389 mg/L; No. 2, 0.704 mg/L; No. 3, 0.974 mg/L; and group No. 4, 1.54 mg/L.)

Gravimetric Aerosol Concentration - Calculated from passing known samples of air collected near animal heads through pre-weighed filters.

Average of 4 samples per concentration

	#1	#2	#3	#4
Mean Concentration (mg/L)	4.19	4.96	6.32	11.33

Nominal Aerosol Concentration - Total column of air passed through exposure chamber divided into total amount of test material expended.

	#1	#2	#3	#4
Mean Concentration (mg/L)	11.5	18.1	27.9	58.4

* Particle size determination:

Dose #1

Mass median aerodynamic diameter = 2.693 micrometers
Geometric Standard Deviation = 2.250

Dose #2

Mass median aerodynamic diameter = 2.815 micrometers
Geometric Standard Deviation = 2.163

Dose #3

Mass median aerodynamic diameter = 3.259 micrometers
Geometric Standard Deviation = 2.295

Dose #4

Mass median aerodynamic diameter = 3.041 micrometers
Geometric Standard Deviation = 2.356

* 85% of the particles were measured to be 10 microns or less

Mortality (using the analytically determined dose levels)

Quoted from the tester's report:

%	Dose (mg/L)	Dead	Treated	Corrected Mortality %	M	F
	0.389	0	10	0	0/5	0/5
	0.704	1	10	10	0/5	1/5
	0.974	6	10	60	5/5	1/5
	1.540	10	10	100	5/5	5/5

End of quotation.

LC_{50} determined by the method of Litchfield & Wilcoxon = 0.9171 mg/L (95% C.L. of .7711 to 1.0907 mg/L).

Pharmacologic/toxic effects included: activity decrease, aggressiveness, alopecia, ataxia, constricted pupils, diarrhea, dilated pupils, emaciation, lacrimation, nasal discharge, piloerection, polyuria, ptosis, respiratory gurgle, salivation, and sensitive to touch. All animals gained weight with the exception of those that died. (1)

Gross necropsy findings considered to be possibly related to Farnesol administration were noted such as: alopecia, chromodacryorrhea, diarrhea, emaciation, nasal discharge, polyuria, salivation, G.I. tract distended with gas, hair coat matted, discoloration of the liver and lungs, and testes drawn into abdominal cavity.

Conclusions:

The LC_{50} calculations were made using the method of Litchfield and Wilcoxon.

The LC_{50} determined in rats using Farnesol (for males, females, and combined):

		95% C.L. (mg/L)
Males	(mg/L) = 0.828	Undefined
Females	(mg/L) = 1.02	0.723-1.45
Overall	(mg/L) = 0.917	0.771-1.09

Toxicity Category: II

Classification:

Tentatively Core Guideline Data (the registrant must submit the purity and composition of the test material used).

(1) Animals dying at the higher doses generally died within 3 days, with male animals being the more sensitive sex. Increased duration and the number of animals responding was observed with increased

005679

DATA EVALUATION RECORD

Subject: Rat Acute Inhalation Toxicity Study

Test Material: Nerolidol. Clear pale yellow liquid. Percent purity and composition not stated.

EPA File Symbol: [redacted] 53971-E

Accession No.: 264527

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
Houston, TX 77036

Sponsor: Fermone Associates, Ltd.
1523 North Post Oak Road
Houston, TX 77055

Report No./Date: Project No. 4027-86, June 24, 1986

Author: Project Leader, Joseph L. Maedgen

Toxicity Category: II

Classification: Tentatively Core Guideline Data

Materials and Methods:

Twenty male and twenty female Sprague-Dawley rats were divided into four groups of five male and five female rats each. Each group was exposed for a given length of time, to create four different aerosol dose levels (the usual method for generating different inhalation dose levels is by varying test solution concentrations).

The exposure chamber was a 500 liter stainless steel dynamic flow chamber. The aerosol was generated by pumping the test material through a pressure-operated air nozzle. The concentrated aerosol was then diluted with dry filtered air and drawn into the exposure chamber through a critical calibrated orifice.

The concentration of the aerosol in the exposure chamber was determined analytically (by gas chromatography) at least once per hour, gravimetrically once per hour (draw a known volume of aerosol sample from animal head vicinity through a preweighed filter. Divide the measured air volume into the collected aerosol weight to give mg/L), and finally determined nominally by dividing the total weight of expended test material volume by the total volume of air passed through the exposure chamber during a given exposure period.

Particle size determinations were made using an Anderson cascade impactor.

Treated animals were observed for mortality and toxic effects several times on the day of exposure and at least once daily to termination at 14 days. Individual body weights were recorded on days 0, 7, and 14, or at the time of discovery after death. Gross necropsies were conducted on all animals at termination, or on discovery after death.

Results:

The dose levels generated and mortality are presented as follows:

Quoted from the tester's report:

Dead/Treated

Dose (mg/L)	Males	Females	Males & Females Combined	% Mortality
0.423	0/5	0/5	0/10	0
0.752	2/5	0/5	2/10	20
2.470	3/5	5/5	8/10	80
3.450	4/5	5/5	9/10	90

End of quotation.

The acute LC_{50} (overall - male and female) with 95% confidence limits (determined according to the method of Litchfield and Wilcoxin):

LC_{50} = 1.4484 mg/L (95% C.L. of 0.8282 to 2.5331 mg/L). 1/

In-life toxic signs included: activity decrease, alopecia, ataxia, bradypnea, chromodacryorrhea, constricted pupils, corneal opacity, diarrhea, dilated pupils, emaciation, lacrimation, nasal discharge, piloerection, polyuria, ptosis, salivation, swollen face. 2/

Gross necropsy findings considered unusual included: alopecia, chromodacryorrhea, diarrhea, epistaxis, lacrimation, nasal discharge, polyuria, salivation, gastrointestinal tract distended with gas, discoloration of lungs, lungs edematous, nodules on lungs, stomach empty, discoloration of urinary bladder contents.

Conclusions:

Rat acute inhalation LC_{50} using Nerolidol = 1.4484 mg/L (95% C.L. - 0.8282 to 2.5331 mg/L).

Toxicity Category: II

Classification:

Tentatively Core Guideline Data (the registrant must provide the percent purity and composition of the material used in this test).

1/ 85% of the particles were measured to be 10 microns or less. The mass median aerodynamic diameter ranged between 2.25 - 4.17 micrometers.

2/ All animals gained weight with the exception of three test dogs. Animals dying at the higher dose generally died within 3 days with medial bleeding the most consistent. Decreased activity, duration and number of animals was observed with increased dose. Some signs persisted for 14 days. (eg - pilo-erection)

DATA EVALUATION RECORD 005679

Subject: Published Study: Screening of Tobacco Smoke Constituents for Mutagenicity Using the Ames' Test.

Test Material: Two hundred and thirty-nine compounds identified in various phases of tobacco smoke were assayed for mutagenicity. Included in the list of tested compounds was Farnesol. All tested compounds were checked for purity using thin-layer chromatography, gas chromatography and nuclear magnetic resonance (NMR).

EPA File Symbol: [redacted] 53871-E

Accession No.: 264527

Testing Facility: Dept. of Bacteriology, Karolinska Institute S-104-01 Stockholm,

and

Research Department
Swedish Tobacco Co.,
P.O. Box 17007, S-10462 Stockholm.

Sponsor: Swedish Tobacco Co.

Report No./Date: Toxicology [redacted]
Elsevier/North-Holland
Scientific Publishers, Ltd.

Authors: Inger Florin, Lars Rulberg, Margareta Curvall, and Curt R. Engell

Classification: Not Acceptable (see Conclusions below)

Materials and Methods:

005679

Salmonella typhimurium histidine deficient mutants used: TA-98, TA-100, TA-1535, and TA-1537. All compounds were tested both with and without metabolic activation using a liver fraction (S-9) from Aroclor 1254 or methylcholanthrene induced rats.

The experimental protocol states that:

Quoted from the tester's report:

> The viable (bacteria) count was determined;
> The number of spontaneous revertants was measured;
> The presence of rfa-mutation was checked by crystal violet inhibition;
>
> The presence of the plasmid pKM 1010 in strains TA-98 and TA-100 was checked by resistance to ampicillin;
> The response to the positive con ols N-methyl-N'-nitrosoguanidine (not requiring metabolic activation) and 2-aminoanthracene (requiring activation) was checked.

End of quotation.

Each substance was tested at 3 µmol/plate (including Farnesol).

Substances giving an uncertain result in the spot test were tested quantitatively at four concentration levels (0.03, 0.3, 3.0, and 30 µmol/plate).

The testers stated that some of the potential mutagens tested precipitated on the plates, and that "results with these substances are difficult to evaluate."

Results:

The testers stated that, in this experiment, Farnesol was not found to be mutagenic. However, the testers also stated that Farnesol precipitated on the plates.

Conclusions:

Farnesol precipitated on the spot test plates; and the testers stated that those preparations which precipitated in the spot tests "were difficult to evaluate."

The exact concentration of Farnesol tested is assumed to be 3 µmol/plate.

The testers did not state how many replicate plates per concentration tested were used (should be a duplicate set, at least).

The positive and negative control results were not presented.

Proof that the Farnesol concentration tested approached that which demonstrated S. typhimurium cell lethality was not presented.

The above-cited experimental deficiencies preclude acceptance of this Ames mutagenicity study.

Classification:

This study is not acceptable to demonstrate a lack of gene mutation potential for Farnesol.

It was also noted that since there is literature against, raw data was not submitted.