CASWELL FILE

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

005671

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JAN 16 1987

MEMORANDUM

SUBJECT: EPA File Symbol 53871-E, PP#6F3434. Request for Exemption From the Requirement of Tolerances on Raw Agricultural Commodities for Stirrup-M Pheromone Used as a Mite Attractant in Pesticide Formulations (No Accession Numbers)

447AB
Caswell No. ~~801E~~

FROM: William S. Woodrow, Ph.D. WSW 1-16-87
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Hazard Evaluation Division (TS-769C)

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

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 1/16/87

1/16/87

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

Action Requested

Fermone Chemicals requests an exemption from the requirement of tolerances for Stirrup-M mite pheromone for use on all raw agricultural commodities (RAC's) when tank-mixed with EPA-registered miticide pesticides. Woodrow's memorandum of July 9, 1986 to Willie Nelson, PM Team 17, cited the following data gaps for data required to support the use of Stirrup-M on RAC's. These data are necessary to

complete Tier 1 data requirements under a Stirrup-M "biochemical pesticide" designation:

- 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 chromosomal aberrations, direct DNA damage and repair, etc.
- o The surfactant [redacted] contained in the product formulation must be cleared by the Agency for food use.

Woodrow's July 9, 1986 memorandum stated that a general exemption from tolerance for the active ingredients in Stirrup-M may be considered upon submission of all of the outstanding data (cited above), and upon proper verification of food use clearance for [redacted] surfactant inert.

Recommendations

An exemption from the requirement of tolerances for Stirrup-M pheromone tank mixed with registered miticides for use on all RAC's is toxicologically supported.

The Tier I biochemical pesticide data gaps listed for Stirrup-M under Action Requested above (present report) have been successfully completed as follows:

1. Justification for waiving data requirements: Request for one 90-day subchronic feeding and one teratology study.

   40 CFR 158.165(c) states that a requirement for one 90-day subchronic feeding and one teratology study under the Tier I biochemical pesticide tests are conditional requirements; that is, if certain conditions of use/exposure are met, these studies may be required.

   Two characteristics of Stirrup-M, which argue against [redacted] the need for one 90-day subchronic feeding and one teratology study: a lack of intrinsic acute toxicity

(as shown by Tier I biochemical pesticide tests listed below) and a nonexistent or negligible potential for exposure resulting from use.

Stirrup-M lack of acute toxicity [redacted]

	Toxicity Category
Acute oral rat LD_{50} > 5050 mg/kg	IV
Acute dermal rabbit LD_{50} > 2020 mg/kg	III
Acute inhalation LC_{50}, rat > 3.37 mg/L	III
Primary ocular irritation, rabbit; mild ocular irritation	III
Primary dermal irritation, rabbit, slight (transitory irritation)	IV

Mutagenicity tests (reviewed in the present report)

a. Ames Salmonella/Microsome Assay - No mutagenic potential (acceptable test).

b. DNA repair using $PolA^+$ and $PolA^-$ E. coli - no mutagenic potential (acceptable test).

c. Sister chromatid exchange study in Chinese hamster ovary cells - no mutagenic potential (acceptable test).

Limited Exposure Potential [redacted]

The maximum recommended application rate for Stirrup-M is very low; 3.04 grams of active ingredient (a.i.) per acre (A).

The current intent is for use of Stirrup-M on cotton and citrus crops to aid in mite control.

Natural levels of one of the two a.i.'s in Stirrup-M, Nerolidol, are available for orange juice at 0.02 to 0.03 mg/L.

The registrant points out that during the 1984-85 Florida orange growing season, approximately 5207 L of orange juice/A were produced. Using a conservative estimate of 10% of Nerolidol (from Stirrup-M) appearing in Florida orange juice, it may be calculated that 0.026 mg Nerolidol/L [redacted] orange juice [redacted] would be within natural levels.

Thus, the natural background of Nerolidol contained in Florida orange juice would approximate that contributed by Nerolidol application to control mites, using a "worst case example." Thus, it is very unlikely that 10% of Stirrup-M or Nerolidol used to treat oranges would ultimately appear in orange juice.

2. Inert clearance for [redacted] surfactant.

During examination of the various [redacted] ingredients prior to determining clearance for use of this material on food crops, a determination was made to object to its incorporation into the Stirrup-M formulation due to the presence of [redacted] in [redacted]

In a January 7, 1987 letter to Mr. Arturo Castillo, PM 17, Fermone Chemicals stated they will have the [redacted] supplier [redacted] replace all of the [redacted] in [redacted] with [redacted] at the same weight-percent as was used for the [redacted]

Thus, the [redacted] used in Stirrup-M will not contain [redacted] but will contain [redacted] in place of the [redacted]

[redacted] ingredients are cleared as inerts under 40 CFR 180.1001, and the [redacted] is cleared under 40 CFR 180.1001(c).

3. Mutagenicity studies (reviewed in the present report).

a. Salmonella/Microsome (Ames) mutagenicity assay, using a Nerolidol-Farnesol mixture.

No mutagenic potential.

Classification: Acceptable study.

b. DNA repair test with $PolA^+$ and $PolA^-$ E. coli bacteria, using a Nerolidol-Farnesol mixture.

No mutagenic potential.

Classification: Acceptable study.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

c. Sister chromatid exchange assay in Chinese hamster ovary cells, using a Nerolidol-Farnesol mixture.

No mutagenic potential.

Classification: Acceptable study.

4. Justification for exemption from tolerances for Stirrup-M used as a tank mix with registered pesticides on all RAC's to control mites.

a. Both Nerolidol and Farnesol (Stirrup-M active ingredients) have been cleared under 21 CFR 172.515 for use as synthetic food flavoring agents and adjuvants.

b. Stirrup-M active ingredients Nerolidol and Farnesol are naturally occurring terpene alcohols that are widely distributed in nature in plant materials. Apparently, Farnesol concentrations between 30 and 470 ppm have been reported on a dry-weight basis in plant leaves. Both Farnesol and Nerolidol have been reported as natural constituents of fragrant essential oils.

c. The low application rate of 3.04 g/A mixed with registered miticide pesticides suggests that the possibility of human exposure due to Stirrup-M use would be negligible or very remote.

d.* Tier I biochemical pesticide acute toxicity tests demonstrate that Stirrup-M exhibits very low toxicity. In addition, the battery of Stirrup-M mutagenicity tests recently submitted (reviewed this report), show no mutagenic potential.

Previously Submitted Toxicity Data

See Woodrow's July 9, 1986 memorandum to Willie Nelson, PM Team 17.

Data Reviewed in the Present Report

*e Please report and submit all test concentrations in terms of ug/ml and ug/plate

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DATA EVALUATION RECORD

1. Subject: Mutagenicity of Nerolidol-Farnesol Mixture in the Ames Salmonella/Microsome Reverse Mutation Assay

2. Test Material: Mixture of Nerolidol (purity 98.7%) and Farnesol (purity 99.2%) on a weight basis 1:1.22. A bright yellow liquid

3. EPA File No.: 53871E

4. Accession No.: No Accession Numbers

5. Sponsor: Fermone Chemicals, Inc.
305 South 2nd Avenue, Suite 101
Phoenix, AZ 85003

6. Testing Facility: Hazleton Biotechnologies
Landjuweel II
3905 PE Veenendaal
The Netherlands

7. Report No./Date: Genetics Assay No.: E-9566,
October 9, 1986

8. Author: A.J.W. Hoorn

9. Classification: Acceptable

10. Materials and Methods:

The Salmonella typhimurium histidine auxotrophs used were TA-1536, TA-1537, TA-1538, TA-98, and TA-100.

An S9 metabolic activation system was prepared from a 9000 x g supernatant homogenate of Aroclor 1254-induced Sprague-Dawley rat liver.

Minimal agar media plates for the selection of histidine revertants consisted of Vogel Bonner Medium E with 2% glucose and 1.5% bactoagar. Plate overlay agar/100 mL contained 0.6 g purified agar, 10 mL of 0.5 mM L-Histidine, 0.05 mM Biotin, and 0.5 g NaCl.

Positive Controls:

Quoted from the tester's report

Assay	Chemical	Solvent	Concentrations per plate (ug)	Salmonella Strains
Nonactivation	Sodium azide (SA)	Water	10.0	TA-1535, TA-100
	2-Nitrofluorene (NF)	Dimethyl-sulfoxide	10.0	TA-1538, TA-98
	Quinacrine mustard (QM)	Dimethyl-sulfoxide	5.0	TA-1537
Activation	2-Anthramine (2AA)	Dimethyl-sulfoxide	2.5	For all strains

End of quotation

A preliminary range-finding study to determine test dosages was conducted with strain TA-100, using 14 doses from a top dose of 150 uL/plate.

Evaluation of Test Results:

For S. typhimurium strains TA-1535, TA-1537, and TA-1538, a positive mutagenic response would consist of positive results over a minimum of three concentrations of test material, and the increase in revertants to histidine prototrophy is equal to or greater than three times the solvent control values.

For strains TA-98 and TA-100, a positive mutagenic response would consist of positive increases in revertants over a minimum of three test dose levels, and the revertant numbers reach a doubling of the solvent control values at the peak of the dose response.

Solvent control revertant colony numbers generally considered acceptable:

TA-1535:	8-30	Revertant Colonies
TA-1537:	4-30	Revertant Colonies
TA-1538:	10-35	Revertant Colonies
TA-98:	20-75	Revertant Colonies
TA-100:	80-250	Revertant Colonies

Three plates per dose level were employed in the presence and absence of a metabolic activation system and the entire assay was performed twice.

11. Results:

Range-finding cell toxicity test:

Quoted from the tester's report

Test Compound uL/Plate	Number of Colonies/Plate	Appearance of Background Lawn
Solvent Control*	124 & 129	Normal
0.02	147	Normal
0.04	120	Normal
0.07	93	Normal
0.15	86	Reduced
0.29	60	Reduced
0.59	24	Reduced
1.17	11	Reduced
2.34	10	Reduced
4.69	20	Reduced
9.38	26	Reduced
18.75	27	Reduced
37.50	37	Reduced
75.00	22	Reduced
150.00	23	Reduced

*Solvent Control 150 uL of solvent per plate

End of quotation

Note that significant reductions in numbers of TA-100 revertants occurred at a test concentration of 0.07 uL/plate; however, since complete inhibition of bacterial cells did not occur at any of the range-finding dose levels used, the tester decided to conduct the mutagenicity tests at all trial doses through 150 uL/plate.

Mutagenicity Assays:

The five test S. typhimurium histidine auxotroph strains tested against the Farnesol-Nerolidol mixture in the presence and absence of a metabolic activation system did not exhibit any indication of mutagenic potential.

The positive control chemicals induced a marked increase in histidine revertants in the presence and absence of a metabolic activation system.

To demonstrate results using one of the five strains (TA-100) tested, the following data are quoted from the tester's report; this was the test strain used to perform the initial cell toxicity range-finding test:

	Revertants Per Plate				
Activation	1	2	3	Mean	SD
Solvent Control*	128	136	139	134.3	5.7
Positive Control**	2179	2275	2224	2226.0	48.0
Test Compound: (Farnesol-Nerolidol)					
0.1 uL	43	50	70	54.3	14.0
1.0 uL	42	43	16	33.7	15.3
5.0 uL	26	19	34	26.3	7.5
10.0 uL	36	22	34	30.7	7.6
25.0 uL	33	36	39	36.0	3.0
50.0 uL	47	42	30	39.7	8.7
100.0 uL	27	35	38	33.3	5.7
150.0 uL	44	44	24	37.3	11.5

*Solvent Control 150 uL per plate
**2-Anthramine 2.5 ug per plate
SD - Standard Deviation: (σ_{n-1})

Nonactivation	Revertants Per Plate 1	2	3	Mean	SD
Solvent Control*	139	144	130	137.7	7.1
Positive Control**	1126	1072	1068	1088.7	32.4
Test Compound:					
0.1 uL	11	16	14	13.7	2.5
1.0 uL	18	16	8	14.0	5.3
5.0 uL	17	14	16	15.7	1.5
10.0 uL	18	16	17	17.0	1.0
25.0 uL	24	23	17	21.3	3.8
50.0 uL	24	19	17	20.0	3.6
100.0 uL	20	25	23	22.7	2.5
150.0 uL	30	23	27	26.7	3.5

*Solvent Control 150 uL per plate
**TA-98: 2-nitrofluorene 10 ug per plate
TA-100: Sodium azide 10 ug per plate
SD - Standard Deviation: (σ_{n-1})

End of quotation

The test results using the remaining five test strains (TA-1535, TA-1537, TA-1538, and TA-98) also demonstrated a total lack of mutagenic potential when tested against the Nerolidol-Farnesol mixture.

12. Conclusions:

A mixture of Nerolidol and Farnesol tested in the presence and absence of a metabolic activation system did not demonstrate mutagenic potential using the Ames Salmonella/Microsome Assay.

13. Classification: Acceptable study.

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DATA EVALUATION RECORD

1. Subject: Clastogenic Evaluation of a Nerolidol-Farnesol Mixture in an In Vitro Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells.

2. Test Material: 1:1.22 mixture (by weight) of Nerolidol (purity 98.7%) and Farnesol (purity 99.2%). Mixture is a clear, yellow liquid.

3. EPA File No.: 53871-E

4. Accession No.: (None)

5. Sponsor: Fermone Chemicals, Inc.
305 South Second Avenue
Phoenix, AZ 85003

6. Testing Facility: Hazleton Biotechnologies
Landjuweel 11
3905 PE Veendaal
The Netherlands

7. Report No./Date: Genetics Assay No. E-9566, October 1986

8. Authors: Study Director: R.D.F.M. Taalman

9. Classification: Acceptable

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10. Materials and Methods:

Chinese Hamster Ovary (CHO) cells. The cells were obtained from an ovary biopsy of a Chinese hamster. Cells were grown in McCoy's 5a medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics; 0.8 x 10^6 cells/ 80 cm^2 flask.

The metabolic activation system consisted of rat liver enzymes and NADP + isocitric acid. The preparation of rat liver microsomes was S9 fraction from male rats treated with Arochlor 1254.

In a first trial, CHO cultures were exposed to a top dose of 3.0 ug/mL, with .5 log decreasing (3) lower doses of the test material.

In a second trial, doses ranged from 1.0 through 100 ug/mL. Before fixation cultures were examined for degree of confluence and the presence of large rounded (mitotic) cells. Only cultures expected to yield some dividing cells were fixed. Cells were examined at least from the top four doses where results were available.

Cells (exponentially growing) were treated without metabolic activation to predetermined doses of test material. After 2 hours, 5-bromo-2'-deoxyuridine was added (BrdU final conc. at 10 um); incubation continued 23.5 to 23.75 hours. Two and one-half hours before cell harvest, cultures were washed with saline and fresh culture medium added. Metaphase cells were treated with 0.075 M KCl hypotonic solution, and washed 3X in freshly prepared fixative (methanol:acetic acid, 3:1), dropped onto slides and air-dried. At least two cell growth cycles in the presence of BrdU, and without S9 metabolic activation were thus observed for sister chromatid exchange, following staining, removal of BrdU stain, and chromosome replication.

CHO cells were similarly grown and processed in the presence of the S9 rat liver microsome activation mixture.

A solvent control consisted of CHO cells treated with the maximum amount of solvent used in cultures (10.0 ul/10 mL of Dimethylsulfoxide).

Positive controls consisted of CHO cell exposure to Mitomycin-C (MMC) for the nonactivation experiment, and cyclophosphamide (CP) in the metabolic activation experiments. Final concentrations of 2.5, 3.75, 5.0, and 10 ng/mL MMC, and 1.0, 1.5, and 2.0 ug/mL CP were used to induce sister chromatid exchanges.

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Dried CHO cells on slides were stained by a modified Giemsa technique. First, stained for 15 minutes with Hoechst 33258 (5.0 ug/mL in phosphate buffer), and exposed to "black-light" from one 15 watt bulb for 10 minutes for differentiation between chromatids. Finally, slides were stained with Giemsa for 5 to 20 minutes and air-dried.

M2 (metaphase) cells were scored for frequency of SCE per cell and per chromosome; only cells with the number of centromeres equal to the modal number 21 ± 2 (range 19 to 23) were scored. Fifty cells per dose were scored where possible.

Positive result criteria:

a. Twofold increase (in SCE) over background, at a minimum of three dose levels.

b. If a statistically significant increase in SCE occurred at a minimum of 3 dose levels and evidence for a positive dose response was determined.

11. Results:

(Quoted from the tester's report) CONFIDENTIAL
Assay No.: E-9566

Compound: Nerolidol-Farnesol mixture

Without Activation

Trial No.: I

Treatment	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X}$ ± S.E.	% Increase Over Solvent Control
Controls Untreated:	50	0.42	8.62 ± 0.398	
Solvent: 1% DMSO	50	0.36	7.52 ± 0.425	
Positive: Mitomycin-C 5.0 ng/mL	25	2.08	43.00* ± 1.856	472

Test Compound	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X} \pm$ S.E.	% Increase Over Solvent Control
1.0 ug/mL	50	0.39	8.02 ± 0.374	
3.0 ug/mL	50	0.40	8.36 ± 0.350	
10.0 ug/mL	50	0.39	8.08 ± 0.388	
30.0 ug/mL	50	0.52	10.74*± 0.467	43

With Activation

Treatment	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X} \pm$ S.E.	% Increase Over Solvent Control
Controls Untreated:	50	0.49	10.14 ± 0.403	
Solvent: 1% DMSO	50	0.42	8.74 ± 0.367	
Positive: Cyclophos-phamide 1.5 ug/mL	25	2.52	51.56*± 2.330	490

Test Compound	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X} \pm$ S.E.	% Increase Over Solvent Control
1.0 ug/mL	50	0.43	8.86 ± 0.454	
3.0 ug/mL	50	0.48	9.80 ± 0.469	
10.0 ug/mL	50	0.45	9.34 ± 0.431	
30.0 ug/mL	50	0.47	9.70 ± 0.472	

Assay No.: E9566 TRIAL II

Without Activation

Treatment	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X} \pm$ S.E.	% Increase Over Solvent Control
Controls Untreated:	50	0.36	7.62 ± 0.378	
Solvent: 1% DMSO	50	0.40	8.20 ± 0.393	
Positive: Mitomycin-C 2.5 ng/mL	25	0.91	18.92*± 0.902	131

Test Compound	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X} \pm$ S.E.	% Increase Over Solvent Control
2.5 ug/mL	50	0.38	7.88 ± 0.340	
5.0 ug/mL	50	0.39	7.98 ± 0.330	
10.0 ug/mL	50	0.40	8.36 ± 0.359	
25.0 ug/mL	50	0.45	9.40 ± 0.411	

With Activation

Treatment	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X}$ + S.E.	% Increase Over Solvent Control
Controls Untreated:	50	0.43	8.94 ± 0.405	
Solvent: 1% DMSO	50	0.40	8.38 ± 0.394	
Positive: Cyclophos-phamide 1.5 ug/mL	25	1.83	38.04*± 1.248	354

Test Compound	Total Cells Scored	SCE's/ Chromo-some	SCE's/ Cell $\bar{X} \pm$ S.E.	% Increase Over Solvent Control
2.5 ug/mL	50	0.40	8.20 ± 0.351	
5.0 ug/mL	50	0.40	8.26 ± 0.275	
10.0 ug/mL	50	0.44	9.10 ± 0.391	
25.0 ug/mL	50	0.46	9.54 ± 0.430	

* Significantly greater than the solvent control, $p \leq 0.05$.

End of Quotation

12. Conclusions:

a. A twofold increase in top dose (or lower doses) of Nerolidol-Farnesol test material did not occur with or without activation, either at a top dose level of 30.0 ug/mL, or at 25.0 ug/mL, when test results were compared to solvent control results. However a 43 percent increase did occur at the top dose level of 30.0 ug/mL without metabolic activation - at one dose level only.

b. A statistically significant increase did occur at the top dose level (as above) without activation (30.0 ug/mL) only. Thus, a statistically significant increase in SCE (over solvent controls), did not occur over 3 dose levels, and no positive dose response for the test material was observed.

c. Mitomycin-C (used without metabolic activation) stimulated a 472 percent increase in SCE, at a dose level of 5.0 ng/mL. Cyclophosphamide at 1.5 ug/mL stimulated a 490 percent increase in SCE over solvent controls. Thus, positive control chemicals did cause significant increases in SCE, with and without activation.

d. A Nerolidol-Farnesol mixture did not cause significant increases in sister chromatid exchanges when tested in CHO cells.

13. Classification: Acceptable.

DATA EVALUATION RECORD

1. Subject: Mutagenicity Evaluation of a Nerolidol-Farnesol Mixture in the DNA Repair Test With $polA^+$ and $polA^-$ Mutants of Escherichia coli

2. Test Material: Mixture of Nerolidol (purity 98.7%) and Farnesol (purity 99.2%) on a weight basis 1:1.22

3. EPA File No: 53871-E

4. Accession No.: No Accession Numbers

5. Sponsor: Fermone Chemicals, Inc.
305 South Second Avenue
Phoenix, AZ 85003

6. Approved By: Hazleton Biotechnologies
Landjuweel 11
3905 Veenendaal
The Netherlands

7. Reports No./Date: E-9566, October 1986

8. Authors: Study Director: Dr. A.J.W. Hoorn

9. Classification: Acceptable study

10. Materials and Methods:

The indicator microorganisms:

Quoted from the tester's report:

Strain Designation	Additional Mutations Repair	LPS	R Factor	Relevant Characteristics
W3110 (polA$^+$)	-	-	-	Normal repair
p3478 (polA$^-$)	polA	-	-	Repair deficient

End of quotation.

The Escherichia coli (E. coli) strain W3110 polA$^+$ is the parental strain for the p3478 polA$^-$ strain, which is deficient for DNA polymerase I enzyme; any DNA damage will result in growth inhibition. Apparently the DNA polymerase I enzyme plays a pivotal role in several DNA repair pathways. The mutant (p3478 polA$^-$) differs from the parental strain in that it has less than 1 percent of the DNA polymerase activity and is more sensitive to many agents known to react with cellular DNA. Thus, in the presence of appropriate chemical agents, the more sensitive p3478 polA$^-$ strain may be affected, further reducing or eliminating its DNA repair capacity, which can result in a zone of inhibition from a focal point source (agar plates) of added test chemical or mutagen longer than a similar test using the parental E. coli strain (W3110 polA$^+$). The parental polA$^+$ E. coli strain and the mutant polA E. coli strain replicate at the same rate.

Negative and Positive Control Materials:

Quoted from the tester's report:

Assay	Chemical	Solvent	Concentrations/ plate
Nonactivation	Methylmethane sulfonate (MMS)	Water	20 uL
Activation	Dimethyl nitrosamine (DMN)	Dimethyl-sulfoxide	100 uL

End of quotation.

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An S9 Sprague-Dawley rat liver homogenate induced in the rat by Aroclor 1254, served as the metabolic activation agent.

The zones of inhibition produced by the test article with the DNA polymerase I deficient (limited) p3478 polA$^-$ E. coli strain are obtained by subtracting the zone of inhibition of the E. coli polA$^+$ cultures from that of the E. coli polA$^-$ cultures. Any test in which a differential of 4 mm or greater has occurred is considered to have produced a DNA-modifying effect (evidence of mutagenic potential). Thus, the preferential inhibition of the polA strain by a test article is indicative of a mutagenic event involving DNA polymerase I.

11. Results:

Quoted from the tester's report:

Assay I (Assays conducted in duplicate with polA$^+$ and polA$^-$ strains)

Zone of Inhibition in Millimeters

NONACTIVATION	W3110 polA$^+$			p3478 polA$^-$		
SOLVENT CONTROL*	0	0	0	0	0	0
POSITIVE CONTROL**	32	33	33	52	52	51
TEST COMPOUND:						
0.1 uL	0	0	0	0	0	0
1.0 uL	0	0	0	0	0	0
5.0 uL	0	0	0	0	0	0
10.0 uL	0	0	0	0	0	0
25.0 uL	0	0	0	0	0	0
50.0 uL	0	0	0	0	0	0
100.0 uL	16	16	16	15	15	15
150.0 uL	16	16	17	15	16	16

* Solvent 150 uL per plate.
** Methylmethane sulfonate 20 uL/plate.

Zone of Inhibition in Millimeters

ACTIVATION	W3110 polA+			p3478 polA-		
SOLVENT CONTROL*	0	0	0	0	0	0
POSITIVE CONTROL**	13	13	13	19	18	20
TEST COMPOUND:						
0.1 uL	0	0	0	0	0	0
1.0 uL	0	0	0	0	0	0
5.0 uL	0	0	0	0	0	0
10.0 uL	0	0	0	0	0	0
25.0 uL	0	0	0	0	0	0
50.0 uL	0	0	0	0	0	0
100.0 uL	0	0	0	0	0	0
150.0 uL	0	0	0	0	0	0

* Solvent 150 uL per plate.
** Dimethylnitrosamine 100 uL/plate.

Assay II

Zone of Inhibition in Millimeters

NONACTIVATION	W3110 polA+			p3478 polA-		
SOLVENT CONTROL*	0	0	0	0	0	0
POSITIVE CONTROL**	30	26	27	51	50	50
TEST COMPOUND:						
0.1 uL	0	0	0	0	0	0
1.0 uL	0	0	0	0	0	0
5.0 uL	0	0	0	0	0	0
10.0 uL	0	0	0	0	0	0
25.0 uL	0	0	0	0	0	0
50.0 uL	0	0	0	0	0	0
100.0 uL	14	14	13	12	10	12
150.0 uL	15	15	15	14	14	15

* Solvent 150 uL per plate.
** Methylmethane sulfonate 20 uL/plate.

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Zone of Inhibition in Millimeters

ACTIVATION	W3110 polA+			p3478 polA-		
SOLVENT CONTROL*	0	0	0	0	0	0
POSITIVE CONTROL**	16	16	16	19	20	20
TEST COMPOUND:						
0.1 uL	0	0	0	0	0	0
1.0 uL	0	0	0	0	0	0
5.0 uL	0	0	0	0	0	0
10.0 uL	0	0	0	0	0	0
25.0 uL	0	0	0	0	0	0
50.0 uL	0	0	0	0	0	0
100.0 uL	0	0	0	0	0	0
150.0 uL	0	0	0	0	0	0

* Solvent 150 uL per plate.
** Dimethylnitrosamine 100 uL/plate.

End of quotation.

Activated systems - Assays I and II:

Positive control - A positive mutagenic effect on DNA repair systems (DNA polymerase I) was shown by the polA- mutant zones of inhibition exceeding the polA+ by 3 to 5 mm.

Solvent control - All negative.

Test compound - All test concentrations, including the top dose level of 150.0 uL/plate, were negative (for mutagenic potential).

Nonactivated systems - Assays I and II:

Positive control - Marked zones of inhibition were noted for the polA- strain in nonactivated systems, which indicated a distinct positive mutagenic effect in the DNA polymerase I repair system.

Solvent control - All negative.

Test compound - The two highest test compound dose levels (100.0 and 150.0 uL/plate) induced zones of inhibition on both polA+ and polA- plates; however, these zones of inhibition were almost identical in size, which clearly indicates no positive mutagenic effects on the DNA polymerase I system.

The fact that zones of inhibition were induced on the $polA^+$ parental E. coli strain does indicate that the highest test compound doses tested did approach cell cytotoxic levels.

12. Conclusions:

A Nerolidol-Farnesol mixture did not demonstrate mutagenic potential when evaluated using the pol^+-pol^- DNA repair test.

13. Classification: Acceptable study.