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## PRODUCT

Stalosan F

## STUDY TITLE

Acute Inhalation Toxicity Study in Rats – Limit Test

## DATA REQUIREMENTS

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1300 (1998)

## AUTHOR

Daniel J. Merkel, B.S.

## STUDY COMPLETED ON

March 10, 2005

## PERFORMING LABORATORY

Product Safety Laboratories

2394 Highway 130

Dayton, New Jersey 08810

## LABORATORY STUDY NUMBER

16449

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## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: **ARCH ANGEL LLC**

Company Agent:

| | |
|---|---|
| _____ | _____ |
| Name | Title |
| _____ | _____ |
| Signature | Date |

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# GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Stalosan F

This study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA) with the following exception: Specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: _____
Daniel J. Merkel, B.S.
Product Safety Laboratories

3/10/05
Date

Submitter: _____
Signature

_____
Date

Sponsor: _____
Signature

_____
Date

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## QUALITY ASSURANCE STATEMENT

The Product Safety Laboratories' Quality Assurance Unit reviewed this study for adherence to PSL's Standard Operating Procedures, the study protocol, and all applicable GLP standards. This final report was found to be an accurate representation of the work conducted. Records of QA findings are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

QA activities for this study:

| QA Activity | Date Conducted | Date Findings Reported To Study Director And Management |
|---|---|---|
| Protocol review | 1/26/04[1], 2/11/05 | 1/26/04, 2/14/05 |
| In-process inspection: *Day 2 in-life observations* | 12/23/04 | 2/14/05 |
| Raw data audit | 2/11/05 | 2/14/05 |
| Draft report review | 2/11/05 | 2/14/05 |
| Final report review | 3/10/05 | 3/10/05 |

Louise N. Caruso

Louise N. Caruso, B.S.
Quality Assurance Auditor
Product Safety Laboratories

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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# ACUTE INHALATION TOXICITY STUDY IN RATS - LIMIT TEST

| | |
|---|---|
| **PROTOCOL NO.:** | P330 |
| **AGENCY:** | EPA (FIFRA) |
| **STUDY NUMBER:** | 16449 |
| **SPONSOR:** | ARCH ANGEL LLC<br>636 Hampshire, Suite 208<br>Quincy, IL 62301 |
| **TEST SUBSTANCE IDENTIFICATION:** | Stalosan F<br>Lot #1 Batch 63 |
| **TEST SUBSTANCE DESCRIPTION:** | Pinkish powder |
| **DATE RECEIVED:** | November 8, 2004 |
| **PSL REFERENCE NO.:** | 041108-3D |
| **STUDY INITIATION DATE:** | November 15, 2004 |
| **DATES OF TEST:** | December 21, 2004 - January 4, 2005 |
| **NOTEBOOK NO.:** | 04-94: pages 226-264 |

## 1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Stalosan F by the inhalation route.

## 2. SUMMARY

An acute inhalation toxicity test was conducted with rats to determine the potential for Stalosan F to produce toxicity via the inhalation (nose-only exposure) route. Under the conditions of this study, the single exposure acute inhalation $LC_{50}$ of the test substance is greater than 2.07 mg/L in male and female rats.

After establishing the desired generation procedures during pre-test trials, ten healthy rats (5/sex) were exposed to the test atmosphere for 4 hours. Chamber concentration and particle size distributions of the test substance were determined periodically during the exposure period. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days following exposure. Body weights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. The gravimetric chamber concentration was 2.07 mg/L. Based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor, the mass median aerodynamic diameter was estimated to be 2.8 µm.

All animals appeared active and healthy over the entire 14-day observation period following exposure. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

## 3. MATERIALS

### A. Test Substance

The test substance, identified as Stalosan F, Lot #1 Batch 63, was received on November 8, 2004 and was further identified with PSL Reference Number 041108-3D. The test substance was a pinkish powder and was stored at room temperature. Prior to aerosolization, the test substance was ground in a ball mill for 24 hours and then in a coffee mill (Krups, Model 203). Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained in Denmark.

The following information related to the characterization of the test substance was provided by the Sponsor unless otherwise noted:

Composition: not given

pH: 3.5 (as a 1% w/w solution) [1]

Solubility: Slightly soluble in water.

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable.

### B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 Males and 5 Females. Females assigned to test were nulliparous and non-pregnant.

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino.

3.B.4 Age/Body weight: Young adult (8-9 weeks)/males 223-245 grams and females 176-200 grams at experimental start.

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on December 14, 2004.

[1] As determined by Product Safety Laboratories (from PSL study numbers 16446 and 16445 for the active ingredient and pH, respectively).

## 4. METHODS

### A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature Range: 19-23°C

4.A.3 Photoperiod: 12-hour light/dark cycle

4.A.4 Acclimation Period: 7 days

4.A.5 Food: Purina Rodent Chow #5012

4.A.6 Water: Tap water was supplied *ad-libitum* by an automatic water dispensing system except during exposure.

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted at least once a year and the records are kept on file at Product Safety Laboratories.

### B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study 16449, constituted unique identification.

## 5. PROCEDURE

### A. Pre-Test Trials

Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures to achieve, to the extent possible, the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter less than or equal to 4 µm). In these trials, the following adjustments were made in an attempt to achieve these objectives:

| | |
|---|---|
| Air Pressure: | constant |
| Compressed Generator Airflow: | constant |
| Compressed Mixing Airflow: | constant |
| Total Airflow: | constant |
| Motor Setting: | varied |
| Dust Generating System: | constant |
| Cutting Head: | constant |
| Cutting Blade: | constant |

Packing Pressure: constant
Material Preparation: constant

The procedures and aerosolization equipment used in the full test were based on the results of pre-test trial number 2. This provided a chamber concentration of 2.03 mg/L and a mass median aerodynamic diameter of 2.8 µm. The test substance used in trial number 2 (as well as the full test) was ground first for 24 hours in a ball mill and then in a coffee mill then prior to aerosolization.

## B. Inhalation Procedures

The exposure chamber, air supply and equipment used to measure particle size distribution, airflow and chamber concentration were the same as used during the pre-test trials and are described below.

5.B.1 Nose-Only Exposure Chamber: A nose-only inhalation chamber with an internal volume of approximately 6.7 liters (Mini-Nose Only Inhalation Chamber, ADG Developments LTD) was used for exposure. Animals were individually housed in polycarbonate holding tubes which seal to the chamber with an "O" ring during exposure. The base unit terminates the chamber with a 0.5-inch diameter tube for discharged air.

5.B.2 Air Supply: Approximately 30 liters per minute (Lpm) of filtered air was supplied by an air compressor (JUN-AIR, Model #6-15) to the dust generator. An additional 3.3 Lpm of compressed mixing air, supplied using dry filtered air from a compressed air tank (WELCO) which was introduced into the chamber to help uniformly distribute the test atmosphere by creating a vortex at the chamber inlet. Compressed airflow was measured with a Mass Flowmeter (Omega, Model #FMA 5613). Chamber airflow was monitored throughout the exposure period and recorded periodically. Total airflow ranged from 31.6 to 31.9 with a mean of 31.7 Lpm. Based on the volume of the inhalation chamber, this airflow provided approximately 284 air changes per hour during the study.

5.B.3 Ambient Conditions: The exposure tube temperature and relative humidity ranges during exposure were 21-22º C and 41-48%RH, respectively. The room temperature and relative humidity ranges during exposure were 21º C and 32-40%RH, respectively. In-chamber measurements were made with a Humidity-Temperature Indicator (Taylor, Model #5502) and room conditions were measured with a Temperature-Humidity Monitor (Dickson, Model #TH550). Temperature and relative humidity values were recorded every 15 minutes for the first hour of exposure and every 30 minutes thereafter.

5.B.4 Test Substance Preparation: The test substance was processed in a 1.6-gallon urethane-lined milling jar (Abbethane, Paul O'Abbe) with porcelain grinding media (0.5" balls) for 24 hours. After milling, the ground test substance was sieved through a ⅜" polyethylene sieve and further ground in a coffee mill (Krups, Model 203).

5.B.5 Dust Generation: The test substance was aerosolized using a modified Wright Dust Generator driven by a variable speed motor (Dayton, Model #4Z538A). The test substance was packed into the dust container (Wright, Model DF 183) and compressed to 400 lbs/in$^2$ using a lab press (Carver, Model C). The container was then fitted with a stainless steel cutting head (Model DF 194SS) and cutting blade (Model DF 191SS).

Compressed air was supplied to the dust generator at 30 psi. The aerosolized dust was then fed directly into the chamber through the dust outlet assembly.

5.B.6 Chamber Concentration Measurements: Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals. Samples were collected using 25 mm glass fiber filters (GF/B Whatman) in a filter holder attached by ¼ inch tygon tubing to a vacuum pump (Reliance Electric, Model #G557X). Filter papers were weighed before and after collection to determine the mass collected. This value was divided by the total volume of air sampled to determine the chamber concentration. The collections were carried out for 2 minutes at airflows of 4 Lpm. Sample airflows were measured using a Mass Flowmeter (Omega, Model #FMA 5610).

5.B.7 Particle Size Distribution: An eight-stage Andersen cascade impactor was used to assess the particle size distribution of the test atmosphere. Samples were withdrawn from the breathing zone of the animals at two intervals. The filter paper collection stages were weighed before and after sampling to determine the mass collected upon each stage. The aerodynamic mass median diameter and geometric standard deviation were determined graphically using two-cycle logarithmic probit axes.

5.B.8 Exposure Period: The animals were exposed to the test atmosphere for 4 hours and 1 minute. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium ($T_{99}$). The times for 90 and 99% equilibration of the chamber atmosphere were 0.5 and 1.0 minute, respectively. At the end of the exposure period, the generation was terminated and the chamber was operated for a further 15 minutes with clean air. At the end of this period the animals were removed from the exposure tube. Prior to being returned to their cages, excess test substance was removed from the fur of each animal.

## C. Selection of Animals

On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy rats (five males and five females) were selected for test.

## D. Body Weights

Individual body weights of the animals were recorded prior to test substance exposure (initial) and again on Days 7 and 14 (termination).

## E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity and behavioral changes prior to exposure, at least every 30 minutes during exposure, upon removal from the exposure chamber and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma.

### F. Necropsy

All rats were euthanized via $CO_2$ inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

## 6. STUDY CONDUCT

This study was conducted at Product Safety Laboratories, 725 Cranbury Road, East Brunswick, New Jersey 08816. The primary technician for this study was Jasbir Bawa, B.S. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 40 CFR 160: U.S. EPA GLP Standards: Pesticide Programs (FIFRA)

and in accordance with:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.1300 (1998)

## 7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Laboratories Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

## 8. DEVIATIONS FROM FINAL PROTOCOL

None

## 9. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Product Safety Laboratories, is maintained in the Product Safety Laboratories Archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL.

## 10. RESULTS

Details of all pretest exposure trials are described in Tables 1 through 3. A summary of test exposure information is presented in Tables 4 through 6. Individual body weights, and cage-side and necropsy observations are presented in Tables 7 through 9, respectively.

All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. The gravimetric and nominal chamber concentrations were 2.07 and 15.31 mg/L, respectively. Based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor, the mass median aerodynamic diameter was estimated to be 2.8 µm.

All animals appeared active and healthy over the entire 14-day observation period following exposure. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

## 11. CONCLUSION

Under the conditions of this study, the single exposure acute inhalation $LC_{50}$ of Stalosan F is greater than 2.07 mg/L in male and female rats.

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## SIGNATURES

Stalosan F

We, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Daniel J. Merkel, B.S.
Study Director
Product Safety Laboratories

Date 3/10/05

Gary Wnorowski, B.A., M.B.A. JO 3/10/05
President
Product Safety Laboratories

Date 3/10/05

## TABLE 1: PREPARATION AND GENERATION SYSTEM FOR PRE-TEST TRIALS

| | |
|---|---|
| 1. Dust Generator: | Wright (Modified) |
| 2. Drive Motor: | Dayton, Model #4Z538A |
| 3. Air Supply: | Air Compressor (JUN-AIR, Modle #6-15)<br>Dry filtered compressed air (WELCO) |
| 4. Dust Container: | Model DF 183 |
| 5. Cutting Head/Blade: | Stainless steel head, Model DF 194SS<br>Stainless steel blade, Model DF 191SS |
| 6. Chamber: | 6.7 liter (Mini Nose-Only Inhalation Chamber, ADG Developments LTD) |
| 7. Diluent Airflow Measurements: | Mass Flowmeter (Omega, Model #FMA 5613) |
| 8. Grinding Apparatus | 1.6-gallon urethane-lined milling jar (Abbethane, Paul O'Abbe)<br>Krups Coffee Mill, Model 203 |
| 9. Lab Press | Carver, Model C |

## TABLE 2: PRE-TEST EXPOSURE TRIALS

| Trial No. | Compressed Air Pressure (psi) | Compressed Air Volume (Lpm) | Compressed Mixing Air (Lpm) | Total Air Volume (Lpm) | Dust Generator Motor Setting | Chamber Conc. (mg/L) | Particle Size Sampled |
|---|---|---|---|---|---|---|---|
| 1[1] | 30 | 28.4 | 3.3 | 31.7 | 4.0 | 3.91 | No |
| 2[1] | 30 | 28.3 | 3.3 | 31.6 | 3.50 | 2.03 | Yes |

[1] Test substance used after grinding for 24 hours in a ball mill and then in a coffee mill.

## TABLE 3: SUMMARY OF PRE-TEST EXPOSURE TRIALS[1]

| Trial No. | Chamber Concentration (mg/L) | Mass Median Aerodynamic Diameter (µm)[2] |
|---|---|---|
| 2[3] | 2.03 | 2.8 |

[1] See Tables 1 and 2 for details of generation system applicable to each trial.

[2] This figure is an estimation based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor.

[3] Test substance used after grinding for 24 hours in a ball mill and then in a coffee mill.

## TABLE 4: GRAVIMETRIC CHAMBER CONCENTRATIONS

| Sample Number | Time of Sample (hour) | Mass Collected (mg) | Airflow Sampled (Lpm) | Collection Time (min) | Chamber Concentration (mg/L) |
|---|---|---|---|---|---|
| 1 | 0.5 | 16.1 | 4 | 2 | 2.01 |
| 2 | 1 | 16.3 | 4 | 2 | 2.04 |
| 3 | 2 | 16.9 | 4 | 2 | 2.11 |
| 4 | 2.5 | 16.3 | 4 | 2 | 2.04 |
| 5 | 3.5 | 16.8 | 4 | 2 | 2.10 |
| 6 | 4 | 17.1 | 4 | 2 | 2.14 |
| | | | | **Average ± Standard Deviation** | 2.07 ± 0.05 |

## TABLE 5: PARTICLE SIZE DISTRIBUTION

| Stage | Effective Cutoff Diameter (µm) | % of Total Particles Captured (by weight) | Cumulative (%)[1] |
|---|---|---|---|
| **Sample 1** | | | |
| 0 | 9.0 | 5.6 | 94.4 |
| 1 | 5.8 | 9.3 | 85.0 |
| 2 | 4.7 | 7.2 | 77.8 |
| 3 | 3.3 | 14.3 | 63.6 |
| 4 | 2.1 | 27.6 | 36.0 |
| 5 | 1.1 | 25.9 | 10.0 |
| 6 | 0.7 | 7.2 | 2.8 |
| 7 | 0.4 | 2.3 | 0.5 |
| F | 0.0 | 0.5 | 0.0 |
| **Sample 2** | | | |
| 0 | 9.0 | 4.9 | 95.1 |
| 1 | 5.8 | 10.3 | 84.8 |
| 2 | 4.7 | 8.4 | 76.4 |
| 3 | 3.3 | 14.6 | 61.9 |
| 4 | 2.1 | 26.8 | 35.1 |
| 5 | 1.1 | 25.3 | 9.9 |
| 6 | 0.7 | 7.3 | 2.6 |
| 7 | 0.4 | 2.6 | 0.0 |
| F | 0.0 | 0.0 | 0.0 |

[1] Percent of particles smaller than corresponding effective cutoff diameter.

## TABLE 6: SUMMARY OF PARTICLE SIZE DISTRIBUTION

| Sample No. | Time of Sample (hours) | Collection Time (minutes) | Mass Median Aerodynamic Diameter (µm) | Geometric Standard Deviation |
|---|---|---|---|---|
| 1 | 1.5 | 2 | 2.7 | 2.08 |
| 2 | 3 | 2 | 2.8 | 2.00 |

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## TABLE 7: INDIVIDUAL BODY WEIGHTS

| Animal No. | Sex | Body Weight (g) | | |
|---|---|---|---|---|
| | | Initial | Day 7 | Day 14 |
| 9615 | M | 227 | 294 | 356 |
| 9616 | M | 230 | 294 | 361 |
| 9617 | M | 245 | 310 | 365 |
| 9618 | M | 223 | 287 | 346 |
| 9619 | M | 238 | 261 | 328 |
| 9620 | F | 200 | 232 | 258 |
| 9621 | F | 176 | 211 | 235 |
| 9622 | F | 192 | 227 | 252 |
| 9623 | F | 192 | 215 | 231 |
| 9624 | F | 183 | 209 | 240 |

## TABLE 8: INDIVIDUAL CAGE-SIDE OBSERVATIONS

| Animal Number | Findings | Day of Occurrence |
|---|---|---|
| MALES | | |
| 9615-9619 | Active and healthy | CR[1]-14 |
| FEMALES | | |
| 9620-9624 | Active and healthy | CR-14 |

[1] CR - removal from exposure tube

## TABLE 9: INDIVIDUAL NECROPSY OBSERVATIONS

| Animal Number | Tissue | Findings |
|---|---|---|
| MALES | | |
| 9615-9619 | All tissues/organs | No gross abnormalities |
| FEMALES | | |
| 9620-9624 | All tissues/organs | No gross abnormalities |