eurofins | Product Safety Laboratories

PRODUCT

EasyDECON 200-531X

STUDY TITLE

Acute Inhalation Toxicity Study in Rats - Limit Test

DATA REQUIREMENT

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

AUTHOR

Jennifer Durando, B.S.

STUDY COMPLETED ON

June 4, 2008

PERFORMING LABORATORY

Eurofins | Product Safety Laboratories

LABORATORY STUDY NUMBER

24401

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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: **EFT HOLDINGS, INC.**

Company Agent:

KEVIN J. IRVING	VICE PRESIDENT GM
Name	Title
Signature	6/9/08
	Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

EasyDECON 200-531X

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

Study Director:

Jennifer Durando, B.S.
Eurofins | Product Safety Laboratories

Date

Submitter:

Signature

Date

Sponsor:

Signature

6/9/08

Date

QUALITY ASSURANCE STATEMENT

The Eurofins | Product Safety Laboratories' Quality Assurance Unit reviewed this study for adherence to EPSL'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	Mar 7, 2007[1]; May 30, 2008	Mar 7, 2007; May 30, 2008
In-process inspection: *Day 6 in-life observations*	May 8, 2008	May 30, 2008
Raw data audit	May 30, 2008	May 30, 2008
Draft report review	May 30, 2008	May 30, 2008

Rhonda S. Krick, B.S.
Director Quality Assurance
Eurofins | Product Safety Laboratories

June 3, 2008
Date

[1] EPSL'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:	24401
SPONSOR:	EFT HOLDINGS, INC. 1012 Oster Drive, Suite A Huntsville, AL 35816
TEST SUBSTANCE IDENTIFICATION:	EasyDECON 200-531X Lot #T-1003 1) Penetrator 2) Fortifier 3) Booster
DATE RECEIVED:	February 25, 2008
EPSL REFERENCE Nos.:	1) 080225-6D 2) 080225-7D 3) 080225-8D
STUDY INITIATION DATE:	February 26, 2008
DATES OF TEST:	May 2 – 16, 2008
NOTEBOOK NO.:	08-101: pages 164-164B, 165-203

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to EasyDECON 200-531X by the inhalation route.

2. SUMMARY

An acute inhalation toxicity test was conducted with rats to determine the potential for EasyDECON 200-531X to produce toxicity from a single exposure 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 2.09 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 although two animals lost body weight by Day 7, all animals gained body weight through the 14-day observation period. The gravimetric chamber concentration was 2.09 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 2.35 µm.

Following exposure all animals were hypoactive and some exhibited abnormal respiration, alopecia (around eyes/nose) and/or facial staining. However all female rats recovered by Day 7 and male rats by Day 12 and appear active and healthy for the remainder 14-day observation period. No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

Three individual components of the test substance, identified as EasyDECON 200-531X, Lot #T-1003, were received from the Sponsor on February 25, 2008 and further identified as follows:

Name and Part Number	EPSL Reference Number
Penetrator (part 1)	080225-6D
Fortifier (part 2)	080225-7D
Booster (part 3)	080225-8D

The test substance components were stored at room temperature. Immediately prior to application, as per the Sponsors instructions, 49% part 1 by weight, 49% part 2 by weight and 2% part 3 by weight were mixed together by EPSL (See Amendment #1)). The prepared test mixture was considered the test substance and was used within 8 hours of mixing. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by Baum's Castorine, P.O. Box 230, 200 Mathew Street, Rome, NY 13442.

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

Composition: Akyl Dimethyl Benzyl Ammonium Chlorides – 2%
Hydrogen Peroxide – 3.9%
Diacetin – 2%
Other Ingredients – 92.1%

Physical description: Clear liquid

pH: 9.8

Solubility: Soluble in water, methanol, ethanol and acetone.

Stability: Test substance was expected to be stable for the duration of testing. The prepared sample must be used within 8 hours after mixing.

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 234-277 grams and females 196-223 grams at experimental start.

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on April 22, 2008.

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 and Relative Humidity Ranges: 20-23°C and 51-68%, respectively.

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

4.A.4 Acclimation Period: 10 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 regularly and the records are kept on file at Eurofins | 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 24401, 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 between 1 and 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
Pump Setting:	varied
Pump Type:	constant
Tubing Size:	constant
Atomization System:	constant
Fluid Cap:	constant
Air Cap:	constant

The procedures and aerosolization equipment used in the full test were based on the results of pre-test trial number 3 which provided a gravimetric concentration of 2.07 mg/L and a mass median aerodynamic diameter of 2.5 µm.

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 23.4 liters per minute (Lpm) of filtered air was supplied by an air compressor (JUN-AIR, Model #6-15) to the spray atomization nozzle. An additional 2.3 Lpm of compressed mixing air, supplied using dry filtered air from a compressed air tank (Airgas), 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 25.6 to 26.0 with a mean of 25.7 Lpm. Based on the volume of the inhalation chamber, this airflow provided approximately 230 air changes per hour during the study.

5.B.3 Ambient Conditions: The exposure tube temperature and relative humidity ranges during exposure were 21-22°C and 55-60%, respectively. The room temperature and relative humidity during exposure were 21°C and 54-56%, respectively. The measurements inside the exposure tube 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 Atmosphere Generation: The test atmosphere was generated using a ¼ inch JCO atomizer, (Spraying Systems Co.), FC3 fluid cap (Robert Miller Associates) and 70SS air cap (Spraying Systems Co.). Compressed air was supplied at 30 psi. The test substance was metered to the atomization nozzle through size 14 tygon tubing (Master Flex), using a peristaltic pump (Master Flex, Model #7520-35).

5.B.5 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 3 minutes at airflows of 4 Lpm. Sample airflows were measured using a Mass Flowmeter (Omega, Model #FMA-5610).

5.B.6 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.7 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 0.6 and 1.2 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, a group of naive animals (not previously tested) 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

All animals were observed for mortality during the exposure period. The animals were examined for signs of gross toxicity, and behavioral changes upon removal from the exposure tube 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 Eurofins | Product Safety Laboratories, 2394 US Highway 130, Dayton, New Jersey 08810. The primary scientist 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 based on the following testing guideline:

- 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, Eurofins | 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. AMENDMENT TO PROTOCOL

1. As per the Sponsor's instructions prior to the start of the study, the sample for application was prepared by Eurofins | Product Safety Laboratories. The sample was a mixture of the penetrator (49%), fortifier (49%) and booster (2%). This mixture was to be applied within 8 hours of preparation.

2. Due to his unavailability from 5/30/08 through 6/22/08 (vacation), George E. Moore will be replaced by Jennifer Durando as the Study Director of this study. This change was required in order to finalize this report in a timely manner.

9. DEVIATIONS FROM FINAL PROTOCOL

None.

10. 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 Eurofins | Product Safety Laboratories, is maintained in the Eurofins | Product Safety Laboratories Archives. EPSL 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 may request continued archiving by EPSL.

11. 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 although two animals lost body weight by Day 7, all animals gained body weight through the 14-day observation period. The gravimetric and nominal chamber concentrations were 2.09 and 62.06 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 2.35 µm.

Following exposure all animals were hypoactive and some exhibited abnormal respiration, alopecia (around eyes/nose) and/or facial staining. However all female rats recovered by Day 7 and male rats by Day 12 and appear active and healthy for the remainder 14-day observation period. No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

12. CONCLUSION

Under the conditions of this study, the single exposure acute inhalation LC_{50} of EasyDECON 200-531X is greater than 2.09 mg/L in male and female rats.

SIGNATURE

EasyDECON 200-531X

I, 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.

Jennifer Durando, B.S.
Study Director
Eurofins | Product Safety Laboratories

Date

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

1. Pump Model:	Peristaltic pump (Master Flex, Model #7520-35)
2. Tubing for Pump:	Size 14 tygon tubing (Master Flex)
3. Air Supply:	Air Compressor (JUN-AIR, Model #6-15) Compressed air (Airgas)
4. Atomization:	¼ inch JCO Atomizer (Spraying System, Co.)
5. Fluid Cap:	FC3 (Robert Miller Associates)
6. Air Cap:	70 SS (Spraying Systems Co.)
7. Chamber:	6.7 liter (Mini Nose-Only Inhalation Chamber, ADG Developments LTD)
8. Mixing Airflow Measurements:	Mass Flowmeter (Omega, Model #FMA-5613)

TABLE 2: PRE-TEST EXPOSURE TRIALS

Trial No.	Compressed Air Pressure (psi)	Compressed Generator Air (Lpm)	Compressed Mixing Air (Lpm)	Total Air Volume (Lpm)	Pump Setting	Chamber Conc. (mg/L)	Particle Size Sampled
1	30	23.5	2.3	25.8	4.0	0.57	No
2	30	23.4	2.3	25.7	10.0	2.72	No
3	30	23.4	2.3	25.7	8.0	2.07	Yes

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

Trial No.	Chamber Concentration (mg/L)	Mass Median Aerodynamic Diameter (µm)[2]
3	2.07	2.5

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

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

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	24.6	4	3	2.05
2	1	27.6	4	3	2.30
3	2	24.7	4	3	2.06
4	2.5	26.0	4	3	2.17
5	3.5	23.0	4	3	1.92
6	3.75	24.6	4	3	2.05
				Average ± Standard Deviation	2.09 ± 0.13

TABLE 5: PARTICLE SIZE DISTRIBUTION

Stage	Effective Cutoff Diameter (µm)	% of Total Particles Captured (by weight)	Cumulative (%)[1]
Sample 1			
0	9.0	4.5	95.5
1	5.8	7.3	88.1
2	4.7	5.9	82.2
3	3.3	15.0	67.2
4	2.1	16.7	50.6
5	1.1	35.9	14.7
6	0.7	10.7	4.0
7	0.4	3.1	0.8
F	0	0.8	0.0
Sample 2			
0	9.0	2.9	97.1
1	5.8	7.6	89.5
2	4.7	6.3	83.2
3	3.3	14.9	68.3
4	2.1	17.3	51.0
5	1.1	34.8	16.2
6	0.7	11.5	4.7
7	0.4	3.7	1.0
F	0	1.0	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	2.4	2.11
2	3	2	2.3	2.07

TABLE 7: INDIVIDUAL BODY WEIGHTS

Animal No	Sex	Body Weight (g)		
		Initial	Day 7	Day 14
3301	M	277	291	361
3302	M	268	285	354
3303	M	263	236	305
3304	M	234	249	297
3305	M	258	267	330
3306	F	209	208	245
3307	F	196	213	241
3308	F	196	200	228
3309	F	200	201	215
3310	F	223	234	258

TABLE 8: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
MALES		
3301	Hypoactivity	CR[1]-6
	Active and healthy	7-14
3302	Hypoactivity	CR-6
	Rales (dry)	3-4
	Active and healthy	7-14
3303	Hypoactivity	CR-9
	Irregular respiration	3-10
	Rales (dry)	3-11
	Facial staining	3-6, 8-10
	Active and healthy	12-14
3304	Hypoactivity	CR-6
	Rales (dry)	3-5
	Facial staining	8-11
	Active and healthy	7, 12-14
3305	Hypoactivity	CR-6
	Irregular respiration	3-6
	Rales (moist)	3-10
	Facial staining	3-6, 8-11
	Alopecia (around eyes & nose)	9-14
	Active and healthy	12-14

[1] CR - removal from the exposure tube

TABLE 8 (cont.): INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
FEMALES		
3306	Hypoactivity	CR[1]-6
	Irregular respiration, rales (moist) facial staining	3-5
	Active and healthy	7-14
3307	Hypoactivity	CR-5
	Rales (moist)	3-5
	Active and healthy	6-14
3308	Hypoactivity	CR-5
	Rales (moist)	3-4
	Irregular respiration, facial staining	3-5
	Active and healthy	6-14
3309	Hypoactivity	CR-5
	Facial staining	3-5
	Active and healthy	6-14
3310	Hypoactivity	CR-5
	Facial staining	3-5
	Irregular respiration, rales (moist)	3-6
	Active and healthy	7-14

[1] CR - removal from the exposure tube

TABLE 9: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissue	Findings
MALES		
3301 – 3305	All tissues and organs	No gross abnormalities
FEMALES		
3306 – 3310	All tissues and organs	No gross abnormalities