eurofins | Product Safety Laboratories

PRODUCT

EasyDECON 200-531X

STUDY TITLE

Acute Oral Toxicity Up And Down Procedure In Rats

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)

AUTHOR

Jennifer Durando, B.S.

STUDY COMPLETED ON

July 8, 2008

PERFORMING LABORATORY

Eurofins | Product Safety Laboratories

LABORATORY STUDY NUMBER

24399

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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 Irvine — Name; Vice President & GM — Title

Signature — Date: 7/15/08

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

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

7/8/08
Date

Submitter: _____
Signature

_____
Date

Sponsor: _____
Signature

7/15/08
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]; Apr 17, 2008	Mar 7, 2007; Apr 17, 2008
In-process inspection: *Day 9 in-life observations for animal #3102*	Mar 21, 2008	Apr 17, 2008
Raw data audit	Apr 17, 2008	Apr 17, 2008
Draft report review	Apr 17, 2008	Apr 17, 2008

Stephanie Mullin
Stephanie Mullin
Quality Assurance Auditor
Eurofins | Product Safety Laboratories

July 7, 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 ORAL TOXICITY UP AND DOWN PROCEDURE IN RATS

PROTOCOL NO.:	P320.UDP
AGENCY:	EPA (FIFRA)
STUDY NUMBER:	24399
SPONSOR:	EFT HOLDINGS, INC. 1012 Oster Drive, Suite A Huntsville, AL 35816
TEST SUBSTANCE IDENTIFICATION:	EasyDECON 200-531X 1) Lot #T-1003 (Penetrator) 2) Lot #T-1003 (Fortifier) 3) Lot #T-1003 (Booster)
DATE RECEIVED:	February 25, 2008
EPSL REFERENCE NO.:	1) 080225-6D 2) 080225-7D 3) 080225-8D
STUDY INITIATION DATE:	February 26, 2008
DATES OF TEST:	March 11 – 26, 2008
NOTEBOOK NO.:	08-70: pages 118-118B, 119-130

1. PURPOSE

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

2. SUMMARY

An acute oral toxicity test (Up and Down Procedure) was conducted with rats to determine the potential for EasyDECON 200-531X to produce toxicity from a single dose via the oral route. Under the conditions of this study, the acute oral LD_{50} of the test substance (as prepared) is greater than 5,000 mg/kg of body weight in female rats.

An initial limit dose of 5,000 mg/kg was administered to one healthy female rat by oral gavage. Due to the absence of mortality in this animal, two additional females received the same dose level, simultaneously. Since these animals survived, no additional animals were tested. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral

changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration and again on Days 7 and 14 (termination) following dosing. Necropsies were performed on all animals at terminal sacrifice.

All animals survived, gained body weight, and appeared active and healthy during the study. 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 (see Section 8, Amendment #2)

The test substance, identified as EasyDECON 200-531X, Lot #T-1003 (Penetrator), Lot #T-1003 (Fortifier) and Lot #T-1003 (Booster), was received on February 25, 2008 and was further identified with EPSL Reference Numbers 080225-6D, 080225-7D and 080225-8D. The test substance was stored at room temperature. The test substance, as received, was mixed at 49% EPSL #080225-6D, 49% EPSL #080225-7D and 2% EPSL #080225-8D by weight as 100% test substance prior to each administration and was used within 8 hours of mixing (see Section 8, Amendment #2). Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by Baums' Castorine, P.O. Box 230, 200 Matthew 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: 3

3.B.2 Sex: Female, nulliparous and non-pregnant.

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

3.B.4 Age/Body weight: Young adult (10 weeks)/188-200 grams at experimental start.

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on February 26, 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: 14-21°C and 30-54%, respectively. The low temperature reading was due to a temporary malfunction of the environmental control system

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

4.A.4 Acclimation Period: 14 or 15 days

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

4.A.6 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.

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, dose level, 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 number 24399, constituted unique identification.

5. PROCEDURE

A. Selection of Animals

Prior to each dosing, experimentally naive rats were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Three healthy female rats were selected for test.

B. Dose Calculations

Individual doses were calculated based on the initial body weights, taking into account the specific gravity (determined by EPSL) of the test substance (as prepared).

C. Dosing

The prepared test substance was administered using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

Individual animals were dosed as follows:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5,000	S	S
2	3102		S	S
3	3103		S	S

S – Survival

D. Body Weights

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

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. 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 at the end of the 14-day observation period. 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, 725 Cranbury Road, East Brunswick, New Jersey 08816. The primary scientist for this study was Jacek Ochalski, D.V.M. 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 870.1100 (2002)

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. AMENDMENTS TO THE PROTOCOL

1.) 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.

2.) **ADD TO:** The sample for application will be prepared by Eurofins/Product Safety Laboratories. The sample will be a mixture of the penetrator (49%), fortifier (49%) and booster (2%). This must be applied within 8 hours of preparation.

REASON: Sponsor's directions before the start of the study.

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

Individual body weights and doses are presented in Table 1. Individual cage-side and necropsy observations are presented in Tables 2 and 3, respectively.

All animals survived, gained body weight, and appeared active and healthy during the study. 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.

12. CONCLUSION

Under the conditions of this study, the acute oral LD_{50} of EasyDECON 200-531X (as prepared) is greater than 5,000 milligrams per kilogram of body weight in 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

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

7/8/25

Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose[1]
			Initial	Day 7	Day 14	mL
3101	F	5,000	188	200	247	0.89
3102	F		200	219	251	0.94
3103	F		198	220	248	0.93

[1] The test substance, as received, was mixed at 49% EPSL #080225-6D, 49% EPSL #080225-7D and 2% EPSL #080225-8D by weight as 100% test substance prior to each administration. Specific Gravity – 1.060 g/mL.

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
3101 – 3103	Active and healthy	0-14

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissue	Findings
3101 – 3103	All tissues and organs	No gross abnormalities