TRADE SECRET

Study Title

IN-JSE76: Acute Oral Toxicity – Up-And-Down Procedure in Rats

TEST GUIDELINES:	U.S. EPA Health Effects Test Guidelines OPPTS 870.1100 (2002) OECD Guideline for the Testing of Chemicals Test No. 425 (2006)
STUDY AMENDED ON:	February 20, 2009
AUTHOR:	S. Dana Oley, B.A.
STUDY COMPLETED ON:	February 12, 2009
PERFORMING LABORATORY:	Eurofins | Product Safety Laboratories 2394 US Highway 130 Dayton, New Jersey, 08810
LABORATORY PROJECT ID:	DuPont-26932 EPSL Study Number 26453
WORK REQUEST NUMBER:	17562
SERVICE CODE NUMBER:	834
SPONSOR:	E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.

STATEMENTS OF CONFIDENTIALITY

The Following Statement Applies to Countries Other Than the United States of America:

This report is the property of E.I. du Pont de Nemours and Company and contains confidential and trade secret information. Except as required by law, this report should not be partially or fully (i) photocopied or released in any form to an outside party without the prior written consent of E.I. du Pont de Nemours and Company or its affiliates, or (ii) used by a registration authority to support the registration of any other product without the prior written consent of E.I. du Pont de Nemours and Company or its affiliates.

The Following Statement Applies to the United States of America:

STATEMENT OF NO DATA CONFIDENTIALITY

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

Company: E.I. du Pont de Nemours and Company

Company Agent: _____ _____
U.S. Product Registration Manager Date

We have submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consent to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the wording of our marking TRADE SECRET, this marking, by itself, conveys no supplemental claims of confidentiality under FIFRA Sections 10(a) or 10(b). In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material that would have been claimed by the Company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 10(g).

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA FIFRA (40 CFR part 160) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices, except for the items documented below. The items listed did not impact the validity of the study.

The dosing preparations used in the study were not analyzed for stability, homogeneity, or accuracy of concentration. The procedures used by trained staff to prepare the dosing preparations ensured:

- the accuracy of concentration because the test substance and vehicle were weighed on an analytical balance accurate to one to two decimal places,
- homogeneity because the mixture was stirred prior to dosing, and
- stability because each preparation was formulated daily prior to dosing.

Applicant/Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: S Dana Oley 2/20/19
S. Dana Oley, B.A. Date

Applicant/Sponsor: _____ _____
DuPont Representative Date

QUALITY ASSURANCE STATEMENT

The Eurofins | Product Safety Laboratories' Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	Feb 28, 2007[1]; Feb 3, 2009	Feb 28, 2007; Feb 3, 2009
In-process inspection: *Day 7 in-life observations for Animal # 3103*	Nov 20, 2008	Feb 3, 2009
Raw data audit	Feb 3, 2009	Feb 3, 2009
Draft report review	Feb 3, 2009	Feb 3, 2009
Final report review	Feb 12, 2009	Feb 12, 2009

Anselmo Villagran, B.S.
Quality Assurance Auditor
Eurofins | Product Safety Laboratories

February 20, 2009
Date

[1] The protocol used for this study was reviewed by the Quality Assurance group on this date.

CERTIFICATION

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.

S. Dana Oley, B.A.
Study Director
Eurofins | Product Safety Laboratories

2/20/09
Date

AMENDMENT TO FINAL REPORT

Report No.: 26453

Amendment No.: 1

Report Section: Title page

The Service Code Number on the title page of the report was changed from 835 to 834.

Reason:

This change was made to reflect the correct Service Code Number for this study.

AMENDMENT DATE: 2/20/19

AUTHORIZED BY: S. Dana Oley

S. Dana Oley, B.A.
Study Director
Eurofins | Product Safety Laboratories

STUDY INFORMATION

CAS Name (uninverted): 4-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1H-pyrazol-5-yl]carbonyl]amino]-3-methyl-5-[(methylamino)carbonyl]benzoic acid

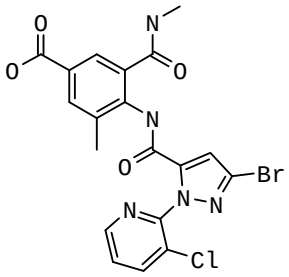
CAS Name (inverted): Benzoic acid, 4-[[[3-bromo-1-(3-chloro-2-pyridinyl)-1H-pyrazol-5-yl]carbonyl]amino]-3-methyl-5-[(methylamino)carbonyl]

IUPAC Name: 4-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)-3-methyl-5-(methylcarbamoyl)benzoic acid

Synonyms/Codes: IN-JSE76 (Report Title)
IN-JSE76 (report text)
IN-JSE76-005 (R&D Lot Number)

Purity: 93.8% by analysis

Structure:



CAS Registry Number: Not available
Molecular Formula: $C_{19}H_{15}BrClN_5O_4$
Molecular Weight: 492.72 g/mole

Known Impurities: None considered to be of toxicological significance at this time.

Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: November 10, 2008 / (see report cover page)

Experimental Start/Termination: November 17, 2008 / December 18, 2008

TABLE OF CONTENTS

IN-JSE76: ACUTE ORAL TOXICITY – UP-AND-DOWN PROCEDURE IN RATS

PROTOCOL NO. (see Section 9):	P320.UDP MOU
AGENCY:	EPA (FIFRA), OECD and JMAFF
STUDY NUMBER:	26453
SPONSOR:	E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.
SPONSOR REPRESENTATIVE:	DuPont Haskell Global Centers for Health and Environmental Sciences Elkton Road, P.O. Box 50 Newark, DE 19714-0050
SPONSOR STUDY MONITOR:	Carol Carpenter
TEST SUBSTANCE IDENTIFICATION:	IN-JSE76 Lot # IN-JSE76-005
DATE RECEIVED:	November 7, 2008
EPSL REFERENCE NO.:	081107-3D
STUDY INITIATION DATE:	November 10, 2008
EXPERIMENTAL INITIATION DATE:	November 17, 2008
EXPERIMENTAL COMPLETION DATE:	December 18, 2008
STUDY COMPLETION DATE:	February 12, 2009
NOTEBOOK NO.:	08-254: pages 247-262

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to IN-JSE76 by the oral route.

2. SUMMARY

An acute oral toxicity test (Up and Down Procedure) was conducted with rats (see Section 9) to determine the potential for IN-JSE76 to produce toxicity from a single dose via the oral route.

Based on a limit dose of 5,000 mg/kg, at the request of the Sponsor, a Main Test was conducted using a default starting dose level of 175 mg/kg administered to one healthy female rat by oral gavage. Following the Up and Down procedure, one female was dosed at 550 and 1,750 mg/kg and 3 females were dosed at 5,000 mg/kg. 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 from each dose level 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.

Under the conditions of this study, the acute oral LD_{50} of the test substance is greater than 5,000 mg/kg of body weight in female rats.

In accordance with the provisions of Directive 1999/45/EC, classification is not required based on the results of this study.

According to the guidance provided by the U.S. EPA and under the conditions of this study, IN-JSE76 is classified in Toxicity Category IV.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

3. MATERIALS

A. Test Substance

The test substance, identified as IN-JSE76, Lot # IN-JSE76-005, was received on November 7, 2008 and was further identified with EPSL Reference Number 081107-3D. The test substance was stored at room temperature. The test sample was administered as a 20% w/w mixture in a 0.5% w/w solution of carboxymethylcellulose (CMC) in distilled water. Preliminary solubility testing conducted by EPSL indicated that mixtures in excess of 20% (i.e. 22.5-60%) were too viscous to be administered. A tissue tearor (Biospec, Model #985370) and a vortex were used to achieve a homogeneous mixture. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor (see Appendix A):

Physical description: Beige solid

pH: 7

Solubility: Not provided.

Stability: The test substance was expected to be stable for the duration of testing

Expiration Date: February 7, 2010

The test substance will be retained for at least 3 months following submission of the final report, unless otherwise specified by the Sponsor. After this time period all remaining test substance will be properly disposed. Records of sample disposition are maintained by EPSL.

B. Animals

3.B.1 Number of Animals: 6

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

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino. (see Section 9)

3.B.4 Age/Body weight: Young adult (12 weeks)/202-234 grams at experimental start.

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on October 21 and November 4, 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: 18-21ºC and 30-67%, respectively.

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

4.A.4 Acclimation Period: 27-30 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 26453, 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). Six 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) and concentration of the test mixture.

C. Dosing

The test substance was administered as a 20% w/w mixture in a 0.5% w/w solution of CMC in distilled water using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Due to the high volume of test suspension to be administered (25.69 mL/kg) at the 5,000 mg/kg dose level, each animal's dose from this group was divided into two approximately equal portions, administered two hours apart. Following the last administration, each animal was returned to its designated cage. Feed was replaced within 3-4 hours after completion of dosing.

Individual animals were dosed as follows:

Main Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	175	S	S
2	3102	550	S	S
3	3103	1,750	S	S
4	3104	5,000	S	S
5	3105		S	S
6	3106		S	S

S – Survival

The test substance was administered in sequence to the animals as described above. The decision to proceed with the next animal was based on the survival of the previous animal following dosing. Dose progressions and stopping criteria were determined using the statistical program described in Section 6.

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. Clinical 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. STATISTICAL ANALYSIS

The *Acute Oral Toxicity (Guideline 425) Statistical Program* (Weststat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations and/or LD_{50} and confidence limit calculations.

7. STUDY CONDUCT

This study was conducted at Eurofins | Product Safety Laboratories, 725 Cranbury Road, East Brunswick, New Jersey 08816. The procedures as described in the protocol are based on the following testing guidelines:

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

OECD Guidelines for Testing of Chemicals, Test No. 425 (2006)

The primary scientist for this study was Jacek Ochalski, D.V.M.

8. 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.

9. DEVIATION FROM FINAL PROTOCOL

Due to a scientist error, rats were used for this study instead of mice as required by this protocol. The Sponsor accepted the rat study. All aspects of the study were conducted in a manner appropriate for rats. Therefore, this deviation had no impact on the conclusion of the study.

10. FINAL REPORT AND RECORDS TO BE MAINTAINED

A copy of the signed report, copies of all raw data generated at Eurofins | Product Safety Laboratories and the original signed protocol, will be maintained in the Eurofins | Product Safety Laboratories archives. EPSL will maintain these records for a period of at least five years.

Laboratory-specific or site-specific raw data, such as personnel files and equipment records will be retained by the facility where the work was done.

The original signed final report and raw data and a copy of the protocol will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

11. RESULTS

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

175, 550 and 1,750 mg/kg (1 animal per level) and 5,000 mg/kg (3 animals)

All animals from each dose level 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 IN-JSE76 is greater than 5,000 milligrams per kilogram of body weight in female rats.

In accordance with the provisions of Directive 1999/45/EC, classification is not required based on the results of this study.

According to the guidance provided by the U.S. EPA and under the conditions of this study, IN-JSE76 is classified in Toxicity Category IV.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

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	175	223	260	278	0.20
3102	F	550	234	274	288	0.66
3103	F	1,750	220	269	278	2.0
3104	F	5,000[2]	230	246	274	5.9
3105	F		202	221	250	5.2
3106	F		213	230	263	5.5

[1] The test substance was administered as a 20% w/w mixture in a 0.5% w/w mixture of CMC in distilled water. Specific Gravity – 0.973 g/mL.

[2] Due to the high volume of test suspension to be administered (25.69 mL/kg) at the 5,000 mg/kg dose level, each animal's dose from this group was divided into two approximately equal portions, administered two hours apart.

TABLE 2: INDIVIDUAL CLINICAL OBSERVATIONS

Animal Number	Findings	Day of Occurrence
175 mg/kg		
3101	Active and healthy	0-14
550 mg/kg		
3102	Active and healthy	0-14
1,750 mg/kg		
3103	Active and healthy	0-14
5,000 mg/kg		
3104, 3105, 3106	Active and healthy	0-14

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissue	Findings
175 mg/kg		
3101	All tissues and organs	No gross abnormalities
550 mg/kg		
3102	All tissues and organs	No gross abnormalities
1,750 mg/kg		
3103	All tissues and organs	No gross abnormalities
5,000 mg/kg		
3104, 3105, 3106	All tissues and organs	No gross abnormalities

APPENDIX A: CERTIFICATE OF ANALYSIS

Exygen RESEARCH
3058 Research Drive
State College, PA 16801
Phone: 814-272-1039
Fax: 814-231-1580

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to the GLP regulations. It documents the identity and purity of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Designation of the Certified Material:

IN:	JSE76
Lot(Dash):	005
Stock No.:	4005408

Analytical Data:

Purity:	93.8%
Appearance:	Solid

The Identity of the Certified Material was Established by Use of LC/MS and NMR
The Purity of the Certified Material was Established by Use of HPLC/UV

Date of Last Analysis:	07-February-2007
Storage:	Ambient
Expiration Date:	07-February-2010

Origin of Certified Material:
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
USA

Testing Facility/Performing Laboratory:

Exygen Research	Spectral Data Services, Inc.
3058 Research Drive	818 Pioneer Street
State College, PA 16801	Champaign, IL 61820

Prepared By: _____ 3/22/07
Ty W. Kahler Date
Study Director

Facility Management: _____ 3/22/07
Charles Simons Date
Operations Manager, Exygen Research