TRADE SECRET

Study Title

Indoxacarb (DPX-MP062) 0.22GB: Acute Oral Toxicity Study in Rodents - Up-and-Down Procedure

TESTING GUIDELINES: OECD Guideline for the Testing of Chemicals
Section 4: Health Effects, Number 425 (2001)

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

AUTHOR: Carol Finlay, B.A.

STUDY COMPLETED ON: November 30, 2004

TESTING FACILITY: E.I. du Pont de Nemours and Company
Haskell[SM] Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050
U.S.A.

LABORATORY PROJECT ID: DuPont-15611

WORK REQUEST NUMBER: 15504

SERVICE CODE NUMBER: 834

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

PAGE RESERVED

Statement of Confidentiality

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 the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, and MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283), except for the item documented below. The item listed does 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 was weighed on an analytical balance accurate to 3 decimal places and the vehicle in which the test substance was suspended was accurately measured with graduated pipettes or flasks,
- homogeneity because the mixture was stirred prior to dosing and while portions were removed for dose administration, and
- stability because the time between dose preparation and administration was kept to a minimum (less than 1 hour).

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

Study Director: Carol Finlay 30-Nov-2004
Carol Finlay, B.A. Date
Staff Toxicologist

Applicant / Sponsor: _____ _____
DuPont Representative Date

QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 15504
Study Code Number: 834

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Conduct:	14 Sep 2004	14 Sep 2004	14 Sep 2004
Report/Records:	23 Nov 2004	23 Nov 2004	23 Nov 2004

Reported by: Annet L. Reigel for DMJ — 30 Nov 2004

Donna M. Johnston — Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Anatomic Pathology Evaluation Reported by: Lisa J. Lewis — 24-NOV-2004
Lisa J. Lewis
Associate Scientist
Date

Anatomic Pathology Evaluation Reviewed by: Steven R Frame — 29-Nov-2004
Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P.
Principal Research Pathologist and Research Manager
Date

Reviewed by: Susan A. MacKenzie — 30-NOV-2004
Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T.
Senior Research Toxicologist
Date

Issued by Study Director: Carol Finlay — 30-Nov-2004
Carol Finlay, B.A.
Staff Toxicologist
Date

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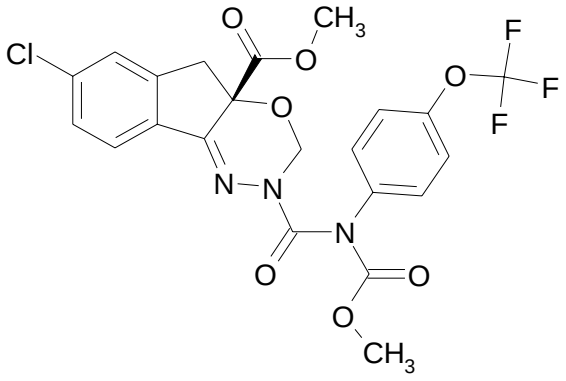
STUDY INFORMATION

CAS Name: (uninverted) Methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl) [4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno [1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylate (for indoxacarb (DPX-MP062) active ingredient)

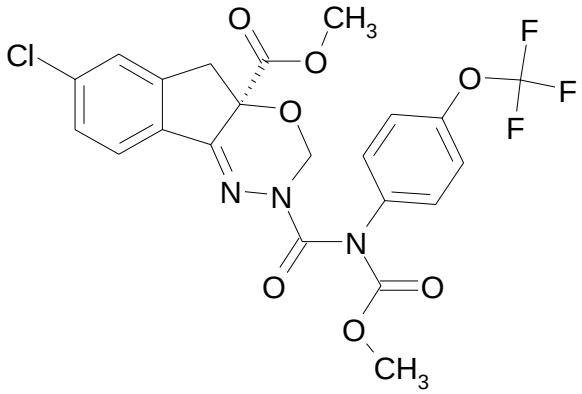
Synonyms/Codes:

- DPX-MP062-397 (Batch Number)
- Indoxacarb (DPX-MP062) 0.22GB
- Indoxacarb 0.22GB
- Indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylic acid, 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]-, methyl ester (inverted CAS name for indoxacarb active ingredient)

Structure: DPX-KN128 S-(+) enantiomer of DPX-MP062



IN-KN127 R-(-) enantiomer of DPX-MP062



Haskell Number: 26633

CAS Registry Number: 144171-61-9 for DPX-MP062
173584-44-6 for the active isomer, DPX-KN128
185608-75-7 for the inactive isomer, IN-KN127

Composition: DPX-MP062-397 is a formulation containing nominal concentrations:

0.22%	DPX-KN128 active ingredient
99.78%	inert ingredients

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: September 10, 2004 / (see report cover page)

In-Life Initiated/Completed: September 14, 2004 / October 5, 2004

SUMMARY

A single dose of Indoxacarb (DPX-MP062) 0.22GB (Indoxacarb 0.22GB) was administered by oral gavage to three fasted female rats at a dose of 5000 mg/kg. The rats were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. All rats were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction.

No deaths occurred and no test substance-related clinical signs were observed. No body weight loss occurred after dosing. No gross lesions were present in the rats at necropsy.

Under the conditions of this study, the oral LD_{50} for Indoxacarb 0.22GB was greater than 5000 mg/kg for female rats.

In accordance with the provisions of Directive 1999/45/EC, which refers to Directive 67/548/EEC for classification criteria, classification is not required.

According to the U.S. EPA, Indoxacarb 0.22GB is classified in Toxicity Category IV.

INTRODUCTION

The purpose of this study was to assess the acute oral toxicity of Indoxacarb (DPX-MP062) 0.22GB (Indoxacarb 0.22GB) when administered by oral gavage to female rats. The starting dose level of 5000 mg/kg was chosen based on available toxicity data.

MATERIALS AND METHODS

A. Test Guidelines

The study design complies with the following test guidelines:

- Organisation for Economic Co-Operation and Development (OECD) (2001). 425 Acute Oral Toxicity – Up-and-Down Procedure. *Guideline for the Testing of Chemicals*.
- Office of Prevention, Pesticides and Toxic Substances (OPPTS) U.S. Environmental Protection Agency (EPA) (2002). OPPTS 870.1100 Acute Oral Toxicity. *Health Effects Test Guidelines*.

B. Test Substance
(Appendix A)

The test substance, Indoxacarb 0.22GB, was supplied by the sponsor as a brown solid. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test Species

Female Crl:CD®(SD)IGS BR rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina.

The Crl:CD®(SD)IGS BR rat was selected based on consistently acceptable health status and on extensive experience with the strain at Haskell Laboratory.

D. Animal Husbandry

1. Housing

Rats were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26°C (targeted to 22-24°C) and a relative humidity of 30-70% (targeted to 40-60%). Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and water were available *ad libitum* except as noted in section E. Dosing.

4. Identification

Each rat was assigned an identification number, which was recorded on a card affixed to the cage. The rats were tail-marked, using a water-insoluble marker, with the identification number.

5. Quarantine

Rats were weighed and observed for general health during the 6-day quarantine period.

6. Animal Health and Environmental Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Dosing

A single oral dose of Indoxacarb 0.22GB, suspended in deionized water, was administered by oral gavage to three fasted female rats at a dose of 5000 mg/kg. One rat was initially dosed. Since this rat survived, two additional rats were then simultaneously dosed the following week. A software package (A0T425StatPgm)[a] was used to determine the dose progression and to calculate the LD_{50}.

The rats were approximately 10 weeks old on the day of dosing. The rats were fasted approximately 17 or 17.5 hours prior to dosing, with food being returned to the rats

[a] Prepared for U.S. EPA by Westat, May 2001, Updated by U.S. EPA February 2002.

approximately 3.5 or 4 hours after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing. The rats were dosed at a volume of 20 mL per kg of body weight. The dosing suspensions were stirred prior to and throughout the dosing procedure.

F. Observations and Body Weights

Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. The rats were observed for clinical signs at the beginning of fasting, just before dosing (test day 0), once during the first 30 minutes after dosing and 3 more times on the day of dosing, and once each day thereafter. The rats were weighed on test days –1, 0, 7, and 14. On test day 14, the rats were euthanized and necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction. The rats were anesthetized by carbon dioxide and euthanized by exsanguination.

RESULTS AND DISCUSSION

In-life Toxicology

A. Dose Progression and Mortality

No deaths occurred. The dose progression and mortality are detailed below.

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Test type: Limit Test
Limit dose (mg/kg): 5000
Assumed LD_{50} (mg/kg): Default
Assumed sigma (mg/kg): 0.5

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	7048	5000	O	O
2	8406	5000	O	O
3	8407	5000	O	O

(X = Died, O = Survived)

Dose Recommendation: The limit test is complete.

SUMMARY OF LONG-TERM RESULTS:

	Dose	O	X	Total
	5000	3	0	3
All Doses		3	0	3

Statistical Estimates:

The LD_{50} is greater than 5000 mg/kg.

B. Body Weights
(Appendices B-C)

No body weight loss occurred after dosing.

C. Clinical Signs
(Appendix D)

No test substance-related clinical signs were observed. Hair loss was observed in one rat but was present at dosing. Hair loss observed in another rat was considered to be incidental.

Anatomic Pathology Evaluation

A. Gross Observations
(Appendix E)

No gross lesions were present in the rats at necropsy.

CONCLUSIONS

Under the conditions of this study, the oral LD_{50} for Indoxacarb 0.22GB was greater than 5000 mg/kg for female rats.

In accordance with the provisions of Directive 1999/45/EC, which refers to Directive 67/548/EEC for classification criteria, classification is not required.

According to the U.S. EPA, Indoxacarb 0.22GB is classified in Toxicity Category IV.

RECORDS AND SAMPLE STORAGE

All data and records for analytical characterizations conducted by or for DuPont Crop Protection will be archived in the DuPont Crop Protection Archives, Newark, Delaware.
Laboratory-specific or site specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

APPENDICES

APPENDIX A

Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898 USA

CERTIFICATE OF ANALYSIS

Characterization is GLP Compliant

TEST ITEM (SUBSTANCE): DPX-MP062-397

LOT NUMBER: E107126-18E

FORMULATION TYPE: Granular Bait (GB)

NOMINAL a.i. CONTENT: 0.22% (indoxacarb)

INSTRUCTIONS FOR STORAGE: Store at Low Humidity, out of direct sunlight at a temperature less than 40 deg. Celsius.

DATE OF ANALYSIS: 12-Aug-04

EXPIRATION DATE: 12-Aug-06

ANALYSIS PERFORMED AT: E. I. du Pont de Nemours and Company
Stine-Haskell Research Center
Newark, Delaware, USA

Reference: DuPont-15344

ANALYTICAL RESULTS:

Active ingredient(s):

ingredient	weight percent
indoxacarb	0.21%

DENSITY: Not applicable to solid formulations.

	NAME	SIGNATURE	DATE
ANALYST	John M. Brisbin	John M. Brisbin	31-Aug-04

APPENDIX B

Individual Body Weights

Individual Body Weights (g)

	Day –1[a]	Day 0[b]	Day 7	Day 14
Female, II 5000 mg/kg				
7048	223.7	203.8	236.5	247.9
Female, IV 5000 mg/kg				
8406	229.8	210.6	245.9	265.1
8407	240.5	220.6	262.4	268.3

a Weight before fasting
b Fasted weight

APPENDIX C

Individual Body Weight Gains

Individual Body Weight Gains (g)

	Days 0-7	Days 7-14	Days 0-14
Female, 5000 mg/kg			
7048	32.7	11.4	44.1
8406	35.3	19.2	54.5
8407	41.8	5.9	47.7

APPENDIX D

Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

Seg - time segment
Prd - predose
pd1 - approximately 0.2 or 0.3 hours postdose
pd2 - approximately 1.75 or 2.5 hours postdose
pd3 - approximately 3.5 or 4 hours postdose
Pd4 - approximately 6 or 6.5 hours postdose

Individual Clinical Observations

Animal	Date	Day	Seg	Observation
7048	13-Sep-2004	-1		General observation, No Abnormality Detected
	14-Sep-2004	0	Prd	General observation, No Abnormality Detected
	14-Sep-2004	0	pd1	General observation, No Abnormality Detected
	14-Sep-2004	0	pd2	General observation, No Abnormality Detected
	14-Sep-2004	0	pd3	General observation, No Abnormality Detected
	14-Sep-2004	0	pd4	General observation, No Abnormality Detected
	15-Sep-2004	1		General observation, No Abnormality Detected
	16-Sep-2004	2		General observation, No Abnormality Detected
	17-Sep-2004	3		General observation, No Abnormality Detected
	18-Sep-2004	4		General observation, No Abnormality Detected
	19-Sep-2004	5		General observation, No Abnormality Detected
	20-Sep-2004	6		General observation, No Abnormality Detected
	21-Sep-2004	7		General observation, No Abnormality Detected
	22-Sep-2004	8		General observation, No Abnormality Detected
	23-Sep-2004	9		General observation, No Abnormality Detected
	24-Sep-2004	10		General observation, No Abnormality Detected
	25-Sep-2004	11		General observation, No Abnormality Detected
	26-Sep-2004	12		General observation, No Abnormality Detected
	27-Sep-2004	13		General observation, No Abnormality Detected
	28-Sep-2004	14		General observation, No Abnormality Detected
				Sacrificed by design
8406	20-Sep-2004	-1		Hair Loss, Forelimb, Right
	21-Sep-2004	0	Prd	Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	21-Sep-2004	0	pd1	Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	21-Sep-2004	0	pd2	Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	21-Sep-2004	0	pd3	Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	21-Sep-2004	0	pd4	Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	22-Sep-2004	1		Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	23-Sep-2004	2		Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	24-Sep-2004	3		Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	25-Sep-2004	4		Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	26-Sep-2004	5		Hair Loss, Forelimb, Right
				Hair Loss, Forepaw, Bilateral
	27-Sep-2004	6		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	28-Sep-2004	7		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	29-Sep-2004	8		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	30-Sep-2004	9		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	01-Oct-2004	10		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	02-Oct-2004	11		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	03-Oct-2004	12		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	04-Oct-2004	13		Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral
	05-Oct-2004	14		Sacrificed by design
				Hair Loss, Forelimb, Bilateral
				Hair Loss, Forepaw, Bilateral

Animal	Date	Day	Seg	Observation
8407	20-Sep-2004	-1		General observation, No Abnormality Detected
	21-Sep-2004	0	Prd	General observation, No Abnormality Detected
	21-Sep-2004	0	pd1	General observation, No Abnormality Detected
	21-Sep-2004	0	pd2	General observation, No Abnormality Detected
	21-Sep-2004	0	pd3	Hair Loss, Sacral, Left
	21-Sep-2004	0	pd4	Hair Loss, Sacral, Left
	22-Sep-2004	1		Hair Loss, Sacral, Left
	23-Sep-2004	2		Hair Loss, Sacral, Left
	24-Sep-2004	3		Hair Loss, Sacral, Left
	25-Sep-2004	4		Hair Loss, Sacral, Left
	26-Sep-2004	5		Hair Loss, Sacral, Left
	27-Sep-2004	6		Hair Loss, Sacral, Left
	28-Sep-2004	7		Hair Loss, Sacral, Left
	29-Sep-2004	8		Hair Loss, Sacral, Left
	30-Sep-2004	9		Hair Loss, Sacral, Left
	01-Oct-2004	10		Hair Loss, Sacral, Left
	02-Oct-2004	11		Hair Loss, Sacral, Left
	03-Oct-2004	12		Hair Loss, Sacral, Left
	04-Oct-2004	13		General observation, No Abnormality Detected
	05-Oct-2004	14		General observation, No Abnormality Detected
				Sacrificed by design

APPENDIX E

Individual Animal Gross Observations

Individual Gross Observations in Female Rats

LESIONS	LESION INCIDENCE Females	
TREATMENT	5000 mg/kg II	5000 mg/kg IV
GENERAL ORGAN	(1)	(2)
NO ABNORMALITY DETECTED	7048	8406 8407

Figures in parentheses are the number of animals grossly examined for this tissue
The absence of a number indicates the finding specified was not identified