European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

INDOXACARB

Volume 3 – B.6 (PPP) – INDOXACARB 150 g/L EC

Rapporteur Member State: France
Co-Rapporteur Member State: Spain

Version History

When	What
2016-12	Initial RAR

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B.6. TOXICOLOGY AND METABOLISM DATA AND ASSESSMENT OF RISKS FOR HUMANS

In the context of the renewal of indoxacarb, the representative formulation Indoxacarb 150 g/L EC is different from the representative formulation of the original DAR.

The test substance specification can be determined from the test substance code which is a research and development code number given to a specific batch of produced material (either technical or formulated). The approximate composition of the material(s) used in the various tests is given in the following table.

Table B.6-1
Test substance specification

Test item	Lot/Batch code	Type	Composition
2014 submission			
Indoxacarb 150 g/L EC	KN128-089	Emulsifiable concentrate (EC)	150 g/L Indoxacarb (DPX-KN128)
Indoxacarb 150 g/L EC	KN128-097	Emulsifiable concentrate (EC)	150 g/L Indoxacarb (DPX-KN128)
Indoxacarb 150 g/L EC	KN128-311	Emulsifiable concentrate (EC)	150 g/L Indoxacarb (DPX-KN128)
Indoxacarb 150 g/L EC	KN128-434	Emulsifiable concentrate (EC)	150 g/L Indoxacarb (DPX-KN128)

B.6.1. ACUTE TOXICITY OF PLANT PROTECTION PRODUCT

Acute toxicity studies were conducted with Indoxacarb 150 g/L EC. Summaries of these studies are presented below.

Table B.6.1-1
Summary of acute toxicity data for Indoxacarb 150 g/L EC

Type of study	Species	Results	References
Acute oral LD_{50}	Rat	LD_{50} = 976.8 mg/kg	[redacted] 2004 DuPont-13455
Acute dermal LD_{50}	Rat	LD_{50} >5000 mg/kg	[redacted] 2003 DuPont 13456
Acute inhalation LC_{50} (4 h)	Rat	LC_{50} >5.2 mg/L	[redacted] 2004 DuPont 13460
Skin irritation	Rabbit	Not irritating	[redacted] 2003 DuPont 13457
Eye irritation	Rabbit	Not irritating	[redacted] 2003 DuPont-13459
Skin sensitisation (Maximisation)	Guinea Pig	Not sensitising	[redacted] 2003 DuPont-13458

Indoxacarb 150 g/L EC had no significant toxicity by the dermal or inhalation routes of exposure. It was harmful by the oral route of exposure. Indoxacarb 150 g/L EC is not an eye or skin irritant and does not cause skin sensitisation.

Taking into account acute toxicity studies, Indoxacarb 150 g/L EC should be classified as Category 4 for acute oral toxicity (H302) according to the provisions of Regulation (EC) No. 1272/2008.

B.6.1.1. Oral

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.1/01

Report: [redacted] (2004); Indoxacarb (DPX-KN128)150 g/L EC: Acute oral toxicity in rodents - up-and-down procedure

DuPont Report No.: DuPont-13455

Guidelines: OECD 425 (2001), OPPTS 870.1100 (2002) **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: DuPont-13455

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
   Lot/Batch #: KN128-097
   Purity: 150 g a.s./L nominal; 151.7 g a.s./L after analysis
   CAS #: None for the formulation
   173584-44-6 for active substance indoxacarb
   Description: Yellow liquid
   Stability of test compound: Not determined. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state, was observed
2. Vehicle and/or positive control: None
3. Test animals
   Species: Rat
   Strain: Crl:CD®(SD)IGS BR
   Age at dosing: Approximately 8-11 weeks old
   Weight at dosing: 186-221.9 g
   Source: [redacted]
   Acclimation period: 6 days
   Diet: PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
   Temperature: 18–26°C
   Humidity: 30–70%
   Air changes: Not recorded
   Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

21-October-2003 to 30-December-2003

2. Animal assignment and treatment

A single oral dose of Indoxacarb 150 g/L EC, as supplied, was administered by intragastric intubation to 5 fasted female rats per dose at doses of 550 or 2000 mg/kg. The animals were dosed one at a time at a minimum of 48-hour intervals. The animals were observed for clinical signs just before dosing, once during the first 30 minutes after dosing and 2 more times within 4 hours after dosing, and once each day thereafter. The animals were weighed on test days –1, 0, 1, 7, and 14. On Test Day 14, surviving animals were euthanized and all animals were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction. A software package (A0T425StatPgm) was used to determine the dose program and to calculate the LD_{50}.

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

Death occurred in 1/4 rats dosed at 550 mg/kg and in 4/5 rats dosed at 2000 mg/kg. Rats were found dead up to 12 days after dosing. The dose progression and mortality are detailed in the tables below.

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

1. Data

Table B.6.1.1-1
Acute oral toxicity of Indoxacarb 150 g/L EC: Dose progression and mortality

Test Seq.	Animal ID	Dose (mg/kg bw)	Short-term result	Long-term result
1	154	550	O	O
2	157	2000	X (Day 3)	X
3	171	550	O	O
4	176	2000	O	O
5	216	2000	X (Day 6)	X
6	178	550	O	O
7	184	2000	X (Day 2)	X
8	185	550	X (Day 12)	X
9	269	2000	X (Day 2)	X

(X = Died, O = Survived)

2. Summary of long-term results

Table B.6.1.1-2
Acute oral toxicity of Indoxacarb 150 g/L EC: Summary of long-term results

Dose (mg/kg bw)	O	X	Total
550	3	1	4
2000	1	4	5
All Doses	**4**	**5**	**9**

(X = Died, O = Survived)

Statistical estimate based on long-term outcomes: The LD_{50} was 976.8 mg/kg bw.

B. CLINICAL OBSERVATIONS

Clinical signs were observed in most rats and included wet or ruffled fur, leaning, prostrate or abnormal posture, ataxia, oral or ocular discharge, hair loss, lung noise, hyper-reactivity, and various staining.

C. BODY WEIGHT

There were no biologically significant, test substance-related body weight effects noted.

D. NECROPSY AND GROSS PATHOLOGY

There were no test substance-related lesions found in this study. The following gross lesions were non-specific and not indicative of target organ toxicity; lung discoloration for study rats 157 (2000 mg/kg) and 185 (550 mg/kg), skin stain for rat 157 (2000 mg/kg), and stomach empty of ingesta and small and large intestines distended with gas for rats 185 (550 mg/kg) and 269 (2000 mg/kg). Study rat 216 (2000 mg/kg) had whole body autolysis.

III. CONCLUSIONS

Under the conditions of this study, the oral LD_{50} was 976.8 mg/kg bw for female rats. In accordance with the provisions of Regulation (EC) No. 1272/2008, Indoxacarb 150 g/L EC is classified as Category 4 for acute oral toxicity.

B.6.1.2. Dermal

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.2/01

Report: [redacted] (2003); Indoxacarb (DPX-KN128) 150 g/L EC: Acute dermal toxicity study in rats

DuPont Report No.: DuPont-13456

Guidelines: 59 NohSan No. 4200 (1985), EEC Method B.3. (1992), OECD 402 (1987), OPPTS 870.1200 (1998) **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: DuPont-13456

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Indoxacarb 150 g/L EC
Lot/Batch #:	KN128-097
Purity:	150 g a.s./L nominal; 151.7 g a.s./L after analysis
CAS #:	None for the formulation 173584-44-6 for active substance indoxacarb
Description:	Yellow liquid
Stability of test compound:	Not determined. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state, was observed
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Age at dosing:	Approximately 9 weeks old
Weight at dosing:	267.0–305.2 g for males; 196.6–214.6 g for females
Source:	[redacted]
Acclimation period:	6 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

14-October-2003 to 28-October-2003

2. Animal assignment and treatment

A dose of 5000 mg/kg body weight was selected for this study. Approximately 24 hours before dosing, the fur of each animal was closely shaved to expose the back from the scapular to the lumbar region (approximately 10% of each animal's body surface area). A single dose of Indoxacarb 150 g/L EC was applied to the intact skin of 5 males and 5 females per dose group. The application site was covered with a porous gauze dressing. After 24 hours, excess test substance was washed from the dorsal skin of each animal with warm water and the skin was dried with a paper towel. Animals were observed for mortality and signs of illness, injury, or abnormal behaviour daily. The animals were observed for clinical signs and dermal irritation daily (weekends excluded). Dermal effects were scored according to the Draize Scale. The animals were weighed on Test Days 0, 1, 7, and 14. On Test Day 14, surviving animals were euthanised and all animals were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction.

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred. Details are provided in the following tableTable .

Table B.6.1.2-2
Acute dermal toxicity of Indoxacarb 150 g/L EC: Doses, mortality/animals treated, dermal LD_{50}

Dose (mg/kg bw)	Males[a]	Females[a]	Combined[a]
5000	0/5	0/5	0/10
Dermal LD_{50}:	**>5000 mg/kg bw**	**>5000 mg/kg bw**	**>5000 mg/kg bw**

[a] Number of animals which died/number of animals in dose group

B. CLINICAL OBSERVATIONS

Red nasal discharge was observed in one rat and red ocular discharges was observed in two rats, the day after application of the test substance, and were attributed to the wrapping procedure. Erythema (score of 1, 2, or 3) was observed in nine rats and oedema (score of 1) was observed in one rat, the day after application. No erythema or oedema was observed by 2 days after application. One rat exhibited epidermal scaling on the test site during the study. No apparent differences between the genders were noted with respect to irritation of the application sites.

C. BODY WEIGHT

Body weight loss of approximately 2-9% of the initial weight occurred in 9 rats the day after application. All rats gained weight during the remainder of the study.

D. NECROPSY AND GROSS PATHOLOGY

No gross lesions were present in the rats at necropsy.

III. CONCLUSIONS

The dermal LD_{50} for Indoxacarb 150 g/L EC was greater than 5000 mg/kg body weight for both male and female rats. In accordance with the provisions of Regulation (EC) No. 1272/2008, classification of Indoxacarb 150 g/L EC by the dermal route is not required.

B.6.1.3. Inhalation

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.3/01

Report: [redacted] (2004); Indoxacarb (DPX-KN128) 150 g/L EC: Inhalation median lethal concentration (LC_{50}) study in rats

DuPont Report No.: DuPont-13460

Guidelines: 59 NohSan No. 4200 (1985), EEC Method B.2. (1992), OECD 403 (1981), OPPTS 870.1300 (1998) **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: DuPont-13460

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S, EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Indoxacarb 150 g/L EC
Lot/Batch #:	KN128-097
Purity:	150 g a.s./L nominal; 151.7 g a.s./L after analysis
CAS #:	None for the formulation 173584-44-6 for active substance indoxacarb
Description:	Yellow liquid
Stability of test compound:	Not determined. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state, was observed
2. Vehicle and/or positive control:	Filtered high pressure air
3. Test animals	
Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Age at dosing:	Approximately 8 weeks old
Weight at dosing:	239–253 g for males; 192–205 g for females
Source:	[redacted]
Acclimation period:	6 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

17-November-2003 to 01-December-2003

2. Animal assignment and treatment

One group of 5 male and 5 female rats were exposed to 5.2 mg/L of the test substance, Indoxacarb 150 g/L EC, suspended in air for a single 4-hour period. During exposure, animals were individually restrained in perforated stainless steel cylinders with conical nose pieces. The restrainers were inserted into a polymethylmethacrylate faceplate attached to the exposure chamber so that the nose of each animal extended into the exposure chamber. Animals were observed for mortality and response to alerting stimuli during the exposure and observed for mortality and clinical signs of toxicity immediately after they were removed from the restrainers following exposure. During a 14-day post exposure period, all surviving rats were observed each day for mortality, and were weighed and observed for clinical signs of toxicity on Test Days 0-8, and 14. At the end of the 14-day recovery period, all surviving animals were necropsied and all animals were examined for gross pathological changes.

3. Generation of the test atmosphere/chamber description

The test substance was metered into a Spraying Systems nebuliser with a Harvard Apparatus Model 22 syringe infusion pump. Filtered, high-pressure air was metered into the nebuliser and carried the resulting atmosphere into the 34-L exposure chamber. The atmospheric concentration of Indoxacarb 150 g/L EC was determined by gravimetric analysis at approximately 30-minute intervals during the exposure period. HPLC analysis was performed on 3 of the samples collected for gravimetric analysis. The filters were desorbed by sonication in acetonitrile and analysed using a Zorbax RX-C8 column and a diode array detector. Samples to determine particle size distribution were taken during the exposure

with a Sierra® Series 210 cyclone preseparator/cascade impactor and Sierra® Series 110 constant flow air sampler. Homogeneous distribution of the test substance in the chamber atmosphere was verified prior to study start.

Table B.6.1.3-1
Acute inhalation toxicity of Indoxacarb 150 g/L EC: Exposure atmosphere characteristics

Parameter	Value
Flow rate	18 L/min
Nominal concentration(s)[a] 120 mg/L	Analytical concentration(s)[b] 5.2 ± 0.14 mg/L
Particle size MMAD[c]/GSD[d]	2.5 µm/1.9
Particles <1 µm (% w/w)	8.0-8.5%
Particles <3 µm (% w/w)	61-62%
Particles <10 µm (% w/w)	98-99%

[a] Theoretical atmospheric concentration calculated when the total amount of test substance delivered to the chamber is divided by the total airflow for the exposure.

[b] Mean was analytically determined from chamber samples.

[c] MMAD = mass median aerodynamic diameter

[d] GSD = geometric standard deviation

4. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were observed in male rats; 2 of the 5 female rats died on Days 5 and 7. Details are provided in the following tableTable .

Table B.6.1.3-2
Acute inhalation toxicity of Indoxacarb 150 g/L EC: Doses, mortality/animals treated, inhalation LC_{50}

Dose (mg/L)	Males[a]	Females[a]	Combined[a]
5.2	0/5	2/5	2/10
Inhalation LC_{50}:	**>5.2 mg/L**	**>5.2 mg/L**	**>5.2 mg/L**

[a] Number of animals which died/number of animals in dose group.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity most often observed in male and female rats were transient and included wet/stained fur, coloured discharge from the nose, and/or postural changes during the recovery period. Other clinical signs of toxicity observed in female rats included lethargy. All surviving animals appeared normal by Day 8 or earlier and throughout the remainder of the study.

C. BODY WEIGHT

One day post-exposure, male rats demonstrated a mean of 7% reduction in body weight and began gaining weight 2 days post-exposure. Female rats lost a mean of 12% in body weight 1 day post-exposure with surviving animals demonstrating weight gains by 4 days post-exposure.

D. NECROPSY AND GROSS PATHOLOGY

No test substance-related gross lesions were present in the rats at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} for Indoxacarb 150 g/L EC in rats was greater than 5.2 mg/L for male and female rats. In accordance with the provisions of Regulation (EC) No. 1272/2008, classification of Indoxacarb 150 g/L EC by the inhalation route is not required.

B.6.1.4. Skin irritation

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.4/01

Report: [redacted] (2003); Indoxacarb (DPX-KN128) 150 g/L EC: Acute dermal irritation study in rabbits

DuPont Report No.: DuPont-13457

Guidelines: OPPTS 870.2500 (1998), EEC Method B.4. (1992), OECD 404 (2002) **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: DuPont-13457

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Indoxacarb 150 g/L EC
Lot/Batch #:	KN128-097
Purity:	150 g a.s./L nominal; 151.7 g a.s./L after analysis
CAS #:	None for the formulation 173584-44-6 for active substance indoxacarb
Description:	Yellow liquid
Stability of test compound:	Not determined. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state, was observed.
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rabbit
Strain:	New Zealand White
Age at dosing:	Young adult
Weight at dosing:	2290–2546 g for males
Source:	[redacted]
Acclimation period:	7 or 13 days
Diet:	PMI® Nutrition International, LLC Certified Rabbit LabDiet® (#5322), approximately 125 g per day
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions	
Temperature:	16-22°C
Humidity:	30-70%
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

23-September-2003 to 20-October-2003

2. Animal assignment and treatment

Indoxacarb 150 g/L EC was applied as a single 0.5 mL dermal dose to the shaved intact skin of 3 male young adult New Zealand White rabbits. One rabbit was initially tested. The remaining two rabbits were treated if there was no severe irritant response in the first animal. The test substance was applied to a 6 cm^2 area of skin. The application area was covered with a 1-inch, 2-ply gauze square that was held in place with non-irritating tape and covered with rubber sheeting for a semi-occlusive dressing. The rabbits were exposed to the test substance for 4 hours after which the test substance was removed. Test sites were evaluated by Draize (1959) for signs of dermal irritation 1, 24, 48, and 72 hours and up to 7 days after test substance removal. The rabbit treated initially was also evaluated immediately after test substance removal.

II. RESULTS AND DISCUSSION

Oedema (score of 1 or 2) was observed in the 3 rabbits one hour after test substance removal. At 24 hours, erythema (score of 1 or 2) was observed in 3 rabbits and oedema (score of 1) was observed in 2 rabbits. At 48 hours, erythema (score of 1 or 2) was observed in 3 rabbits and oedema (score of 1 or 2) was observed in 2 rabbits. No dermal irritation was observed in one rabbit by 72 hours; the remaining two rabbits exhibited erythema (score of 2) and oedema (score of 1 or 2). One of the rabbits also exhibited fissuring of the skin at 72 hours. At 6 days, one rabbit exhibited dry serum and the remaining rabbit exhibited erythema (score of 1) and no dermal irritation at 7 days. According to the study author, dried serum (crusting) is not considered an

adverse dermal effect since it can be removed by washing with water. Therefore, no further evaluations were done for this rabbit. There were no test substance-related body weight effects or clinical signs noted.

Table B.6.1.4-1
Individual dermal irritation scores according to Draize (1959)

Time	Erythema			Oedema		
	32[a,b]	50[a]	44[a]	32[a,b]	50[a]	44[a]
1 h	2	1	2	0	0	0
24 h	2	2	1	1	1	0
48 h	2	2	1	2	1	0
72 h	2[c]	2	0	2	1	0
6 days	1	0[d]	[e]	0	0	[e]
7 days	0	[e]	[e]	0	[e]	[e]

[a] Animal number
[b] Initial rabbit tested
[c] Fissuring
[d] Dry serum
[e] Not scored

Table B.6.1.4-2
Mean individual dermal irritation scores according to Draize (1959)

Animal number	Erythema[a]	Oedema[a]
32	2	1.67
50	2	1
44	0.67	0

[a] Mean of 24 h, 48 h, and 72 h readings

III. CONCLUSIONS

Based on the mean degree of skin reaction observed at 24 to 72 hours, and according to the provisions of Regulation (EC) No. 1272/2008, Indoxacarb 150 g/L EC is not classified for skin irritation.

B.6.1.5. Eye irritation

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.5/01

Report: [redacted] (2003); Indoxacarb (DPX-KN128) 150 g/L EC: Acute eye irritation study in rabbits

DuPont Report No.: DuPont-13459

Guidelines: OECD 405 (2002), EEC Method B.5. (1992), OPPTS 870.2400 (1998) **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: DuPont-13459

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Indoxacarb 150 g/L EC
	Lot/Batch #:	KN128-097
	Purity:	150 g a.s./L nominal; 151.7 g a.s./L after analysis
	CAS #:	None for the formulation 173584-44-6 for active substance indoxacarb
	Description:	Yellow liquid
	Stability of test compound:	Not determined. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state, was observed.
2.	Vehicle and/or positive control:	None
3.	Test animals	
	Species:	Rabbit
	Strain:	New Zealand White
	Age at dosing:	Young adult
	Weight at dosing:	2394–3055 g for males
	Source:	[redacted]
	Acclimation period:	7 days
	Diet:	PMI® Nutrition International, LLC Certified Rabbit LabDiet® (#5322), approximately 125 g per day
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4.	Environmental conditions	
	Temperature:	16-22°C
	Humidity:	30-70%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   20-October-2003 to 31-October-2003

2. Animal assignment and treatment

   A single dose of 0.1 mL Indoxacarb 150 g/L EC was administered into the lower conjunctival sac of the right eye of 3 male young adult New Zealand White rabbits. One rabbit was initially treated. The remaining 2 rabbits were treated if there was no severe irritant response in the first animal. The eyes were not rinsed after introduction of the test substance. The conjunctiva, iris, and cornea of each treated eye were evaluated for evidence of irritation approximately 1, 24, 48, and 72 hours following administration of the test substance using the Draize scale.

II. RESULTS AND DISCUSSION

One rabbit pawed the treated eye after instillation of the test substance. The test substance produced conjunctival redness (score of 1) in 2 rabbits, conjuctival chemosis (score of 1) in 3 rabbits, and discharge (score of 1 or 3) in 3 rabbits. Signs of irritation completely resolved in 1 rabbit by 24 hours and by 72 hours in the remaining 2 rabbits. There was no evidence of corneal injury in any of the treated rabbits. There were no test substance-related body weight effects or clinical signs noted.

Table B.6.1.5-1
Individual eye irritation scores according to Draize (1959)

Cornea			
Animal no.	**41[a]**	**56**	**55**
1 hour[a]	0	0	0
24 hours	0	0	0
48 hours	0	0	0
72 hours	0	0	0
Iris			
Animal no.	**41[a]**	**56**	**55**
1 hour[a]	0	0	0
24 hours	0	0	0
48 hours	0	0	0
72 hours	0	0	0
Conjunctiva-redness			
Animal no.	**41[a]**	**56**	**55**
1 hour[a]	1	1	0
24 hours	1	1	0
48 hours	1	1	0
72 hours	0	0	0
Conjunctiva-chemosis			
Animal no.	**41[a]**	**56**	**55**
1 hour[a]	1	1	1
24 hours	1	1	0
48 hours	0	0	0
72 hours	0	0	0
Conjunctiva-discharge			
Animal no.	**41[a]**	**56**	**55**
1 hour[a]	3	3	3
24 hours	0	1	0
48 hours	1	0	0
72 hours	0	0	0

[a] Initial animal tested

Table B.6.1.5-2
Mean individual eye irritation scores according to Draize (1959)

Animal number	**Corneal opacity[a]**	**Iritis[a]**	**Conjunctival redness[a]**	**Conjunctival chemosis[a]**
41	0.00	0.00	0.67	0.33
56	0.00	0.00	0.67	0.33
55	0.00	0.00	0.00	0.00

[a] Mean of 24 h, 48 h, and 72 h readings.

III. CONCLUSIONS

Based on the mean degree of eye irritation observed at 24 to 72 hours, and according to the provisions of Regulation (EC) No. 1272/2008, classification of Indoxacarb 150 g/L EC is not required.

B.6.1.6. Skin sensitization

Previous evaluation:	Submitted for the purpose of renewal

CP 7.1.6/01

Report: [redacted] (2003); Indoxacarb (DPX-KN128) 150 g/L EC: Dermal sensitization – Magnusson-Kligman maximization method

DuPont Report No.: DuPont-13458

Guidelines: OECD 406 (1992), OPPTS 870.2600 (1998) **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: 14379

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Indoxacarb 150 g/L EC
	Lot/Batch #:	KN128-097
	Purity:	150 g a.s./L nominal; 151.7 g a.s./L after analysis
	CAS #:	None for the formulation 173584-44-6 for active substance indoxacarb
	Description:	Yellow liquid
	Stability of test compound:	Not determined. The test substance was expected to be stable for the duration of the testing.
2.	Vehicle	Distilled water
	Positive control:	α-hexyl-cinnamaldehyder
3.	Test animals	
	Species:	Guinea pig
	Strain:	Hartley albino
	Age at dosing:	Young adult
	Weight at dosing:	268–443 g for males
	Source:	[redacted]
	Acclimation period:	7 days
	Diet:	Pelleted Purina Guinea Pig Chow (#5025), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Animals were group housed in stainless steel cages with wire-mesh floors suspended above cage boards
4.	Environmental conditions	
	Temperature:	18-23°C
	Humidity:	36-61%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

13-October-2003 to 15-November-2003

2. Animal assignment and treatment

Range finding study: Preliminary irritation testing was performed on 14 animals to determine appropriate concentrations of Indoxacarb 150g/L EC that could be used for both intradermal and topical induction as well as topical challenge. The concentration selected for the intradermal induction was a 5% w/w mixture in distilled water. A dose of 100% was found to produce faint to moderate dermal irritation and was selected for the topical induction; and 1% w/w mixture in distilled water did not produce dermal irritation and was selected as the highest non-irritating concentration.
Main study: The first induction phase involved 6 intradermal injections into the suprascapular area of each of the 20 animals on Day 1. The doses were comprised of pairs of injections of the test substance (5% w/w mixture in distilled water), test substance combined with Freund's Complete Adjuvant (5% w/w mixture of test substance in adjuvant), and adjuvant alone (50% v/v adjuvant in distilled water). Approximately one week later, animals were topically induced with 0.5 mL of the test substance (100%) for 48 hours. Animals were challenged on test Day 22 with 0.5 mL of a 1% w/w mixture of the test substance in distilled water and 0.5 mL of a 0.33% w/w mixture of the test substance in distilled water on two separate test sites. A separate group of 10 guinea pigs were similarly challenged with 1 or 0.33% of the EC formulation ingredients (not containing the test substance). An addition group of 10 guinea pigs was challenged with the positive control substance α-hexyl-cinnamaldehyde. Approximately 24 and 48 hours after the challenge phase, the test sites were evaluated for signs of elicited sensitisation.

II. RESULTS AND DISCUSSION

Twenty-four hours after the challenge application, 9 of the 20 guinea pigs treated with 1%; and 3 of the 20 treated with 0.33% of the test substance had very faint erythema scores (score of 0.5). Forty-eight hours after the challenge, 6 of the 20, and 3 of the 20 guinea pigs treated with 1 or 0.33%, respectively, had erythema scores of 0.5.

Twenty-four hours after the challenge with the EC formulation control, 4 of 10 and 0 of 10 guinea pigs treated with 1 or 0.33%, respectively had erythema scores of 0.5. Forty eight hours after the challenge, 2 of 10 and 0 of 10 guinea pigs treated with 1 or 0.33%, respectively, had erythema scores of 0.5.

Twenty-four hours after the challenge with the positive control substance, the erythema scores ranged from 0.5 to 3, with 8 of the 10 having a score of 1 or greater, demonstrating the ability of the test system to detect a sensitizer.

On the basis of this study, Indoxacarb 150 g/L EC does not warrant classification as being a dermal sensitiser.

Table B.6.1.6-1
Maximisation test with Indoxacarb 150 g/L EC: Dermal response 24 and 48 hours after challenge

	Indoxacarb 150 g/L EC group				**EC formulation control group**			
	1%		**0.33%**		**1%**		**0.33%**	
Hours	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
1% w/w mixture of Indoxacarb 150g/L EC in distilled water (0.5 mL)	0/19[a]	0/19	0/19[a]	0/19	0/10	0/10	0/10	0/10

[a] Number of animals with positive dermal response/number of animals in dose group

III. CONCLUSIONS

Based on the absence of skin scores greater than 0.5, and according to the provisions of Regulation (EC) No. 1272/2008, classification of Indoxacarb 150 g/L EC for skin sensitization is not required.

B.6.1.7. Supplementary studies on the plant protection product

28- and 90-day rat studies conducted with the formulation Indoxacarb 150 g/L EC were provided in the context of this renewal. The applicant submitted the following statement: "*Vertebrate studies were undertaken with Indoxacarb 150 g/L EC due to the observation of mortality observed in 90-day studies in female rats previously reviewed and found in the Indoxacarb DAR, Volume 3, B6, 2000, with DPX-MP062 (a mixture of the active substance indoxacarb (DPX-KN128) and its R-enantiomer (IN-KN127), in a 75:25 ratio) at dietary concentrations less than 10 mg/kg/day. Test substance-related mortality occurred in female rats administered 6.09 or 8.94 mg/kg/day of DPX-MP062 in the 90-day neurotoxicity study (HLR 1116-96, Revision No. 1), and in the 90-day study (HL-1997-00056, Revision No. 1). However, test substance-related mortality was not observed in rats administered with DPX-JW062, a racemic mixture of indoxacarb (DPX-KN128) and R-enantiomer (IN-KN127), (HL-1998-01200 and HLR 751-93, Revision No. 2) for 90 days or in rats administered with indoxacarb (DPX-KN128) for 90 days (HLR 301-94, Revision No. 1). Please refer to Indoxacarb EU Renewal Dossier, Document M-CA, Section 5, DuPont-41109 EU for further explanation of DPX-MP062 and DPX-JW062. Mortality was generally observed between test days 8-21, and the cause of mortality was not identified from the pathological evaluations. The above studies are either summarised in Indoxacarb DAR, Volume 3, B.6, 2000 or in Indoxacarb EU Renewal Dossier, Document M-CA, Section 5, DuPont-41109 EU. In order to determine if vertebrate studies were needed to evaluate the potential for mortality from repeated exposure to Indoxacarb 150 g/L EC formulation, DuPont evaluated the available literature for repeated-dose toxicity information on each of*

the components in the formulation, by searching for any repeated dose toxicity data on similar types of mixtures, and evaluating whether in vitro and QSAR methods available at that time could be used to predict the outcome of the studies. Following this in depth review of the available information, sub chronic 90-day vertebrate studies were undertaken with Indoxacarb 150 g/L EC for the following reasons:

1. *Repeated dose toxicity data were not available for several formulation components.*

2. *Available toxicity data for the formulation components indicated that some formulation components could alter gastrointestinal absorption kinetics following repeated dose exposure.*

3. *Results from in vitro assays that could be used for evaluation of gastrointestinal absorption, and in vitro assays that could be used to measure the toxicity of pesticide adjuvants are difficult to interpret due to cytotoxicity, and low water solubility of the adjuvants.*

4. *Results from (Q)SAR models are difficult to apply quantitatively to repeated-dose toxicity endpoints to determine potential concentrations at which effects may be observed. Since the classification criteria is dependent on concentration, (Q)SAR models which could only provide a qualitative assessment at best, could not be used to determine if the concentration criteria for classification have been met."*

28-day study with the formulation Indoxacarb 150 g/L EC:

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.7/01

Report: [redacted] (2012a); Indoxacarb (DPX-KN128) 150 g/L EC (pure active substance indoxacarb) Repeated-dose oral toxicity 28-day feeding study in rats

DuPont Report No.: DuPont-33601

Guidelines: OPPTS 870.3050 (2000), OECD 407 Section 4 (2008), EEC Method Part B7 96/54/EEC (1996)
Deviations: Only females were used on study since they are more sensitive sex; Clinical pathology measurements were limited to hematology parameters, since these were the parameters affected in previous studies with indoxacarb; Microscopic pathology was evaluated only in the target organs identified in previous studies with indoxacarb and for gross lesions identified at necropsy.

Testing Facility: [redacted]

Testing Facility Report No.: DuPont 33601

GLP: Study not required to be in compliance with GLP standards. However, work was conducted in a GLP-compliant facility following Standard Operating Procedures.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Indoxacarb 150 g/L EC
Lot/Batch #:	KN128-311/ JUL10BL024
Purity:	15.1% (143.0 g/L) indoxacarb active ingredient, 84.9% inert ingredients
Description:	Light yellow/amber liquid
CAS #	None for the formulation 173584-44-6 for active substance indoxacarb
Stability of test compound:	Analyses confirmed that test material was stable in feed.
2. Vehicle and/or negative control:	Acetone and untreated diet
3. Test animals	
Species:	Rat
Strain:	Crl:CD(SD)
Age at initial dosing:	Approximately 7 weeks old
Weight at initial dosing:	154.8–270.6 g
Source:	[redacted]
Acclimation period:	7 days
Diet (or dosing solution, if a gavage study):	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed in pairs in solid-bottom caging with bedding mixed with appropriate species-specific enrichment.
4. Environmental conditions	
Temperature:	20–26°C
Humidity:	30–70%
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   07-October-2011 to 02-April-2012

2. Animal assignment and treatment

   Five groups of 5 animals/concentration were administered Indoxacarb 150 g/L EC in feed daily for at least 28 days. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (NRC 2011).
   Dietary concentrations selected for this study were 225, 450, 675, and 900 ppm of DPX-KN128 150 g/L EC, equivalent to 16.23, 27.66, 42.05 and 48.49 mg/kg bw/d respectively, based on results in the previous studies with indoxacarb. The 2 highest concentrations were thought to possibly result in mortality and were likely to produce body weight and hematology effects. The other 2 concentrations were not expected to produce mortality, but were expected to possibly produce body weight and/or hematology effects, based on the predicted estimated mean daily intake of indoxacarb. These concentrations were selected to assess a dose response for any observed effects and to establish a no-observed-adverse-effect level (NOAEL) for Indoxacarb 150 g/L EC.

3. Diet preparation and analysis

   The test substance, Indoxacarb 150 g/L EC (dissolved in acetone) was added to the rodent diet and thoroughly mixed for a period of time that was adequate to ensure homogeneous distribution in the diet.

The diet preparation was not corrected for the purity of the test substance, nor for the percent of active ingredient in the formulation. The same amount of acetone was added to control diets and similarly mixed for the same period of time. Diets were prepared and stored refrigerated until used. The stability, homogeneity, and concentration of Indoxacarb 150 g/L EC in the dietary mixtures were checked by analysis using HPLC with UV detection at the beginning of the study, and concentration was verified during the first month and near the middle and end of study. The test substance was at target concentrations (±17% of nominal), homogeneously mixed (RSD ≤7%) throughout the feed, and was stable (±8% of nominal) for up to 22 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Significance was judged at $p < 0.05$.

Table B.6.1.7-1
Statistics: 90-day feeding study in female rats

Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain Food Consumption Food Efficiency Clinical Pathology Organ Weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with those bilirubin data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with those data.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE STABILITY ANALYSES

Analytical results demonstrate that the test substance was homogeneously mixed, at the targeted concentrations, and stable in the diet under the storage conditions of the study.

A. OBSERVATIONS

1. Clinical signs of toxicity

Transient clinical signs of toxicity were observed, including abnormal gait, ataxia, hunched or high posture, reduced muscle tone and hyperactivity. These signs were generally only seen in a few animals, primarily in the 675 and 900 ppm groups. Except for high posture, these signs were only observed in surviving rats over the first week.

2. Mortality

One rat in the 900 ppm group was sacrificed on test day 11, due to severe body weight loss. The cause of death was bone marrow atrophy/depletion which was considered test substance related.

B. BODY WEIGHTS AND BODY WEIGHT GAINS

Adverse, test substance-related reductions (compared to control) in mean body weight and body weight gain were observed in rats exposed to 450 ppm and above in the diet. Although the difference in final body weight (test Day 28) was not statistically significant in the 450 ppm group, it was considered to be test substance related based on the magnitude of difference (15% lower than control) and a statistically significant overall (test Day 0-28) lower body weight gain (-49.2%).

The body weight effects in these groups were due primarily to body weight loss at 675 and 900 ppm and low (1 gram) body weight gain at 450 ppm during the first week of dietary exposure. Body weight gain in these groups was not statistically significantly different from control over the remaining 3 weeks of exposure. All rats at in the 675 and 900 ppm groups and two rats in the 450 ppm group lost weight over the first week. One rat in each of the 450 and 675 ppm groups also lost weight over the second week. However, all rats in these groups gained weight over the remaining weeks and body weight gain in these groups occasionally exceeded that of control (not statistically significant).

In the 225 ppm group, mean weekly and overall body weight gain was generally lower than in control, but the differences were not statistically significant. No rats in the control or 225 ppm groups lost weight over any week.

Table B.6.1.7-2
Mean body weight gain in grams female rats

Diet		**0 ppm**	**225 ppm**	**450 ppm**	**675 ppm**	**900 ppm**
Days relative to start date:		**1**	**2**	**3**	**4**	**5**
0 - 7	Mean	22.0	17.5	1.0*	-28.7*	-28.1*
	SD	5.3	6.3	6.0	14.8	8.3
	N	5	5	5	5	5
	% Diff		-20.5	-95.3	-230.6	-227.7
7 - 14	Mean	18.5	13.8	14.3	17.6	18.9
	SD	5.5	5.0	9.6	25.1	12.8
	N	5	5	5	5	4
	% Diff		-25.7	-23	-5.1	2.2
14 - 21	Mean	20.7	22.3	16.8	28.7	20.3
	SD	8.1	12.5	6.2	10.6	13.8
	N	5	5	5	5	4
	% Diff		7.4	-19.2	38.2	-2.2
21 - 28	Mean	20.0	18.6	9.2	17.8	11.5
	SD	9.3	6.7	5.5	10.2	10.6
	N	5	5	5	5	4
	% Diff		-6.9	-53.9	-10.9	-42.3
0 - 28	Mean	81.2	72.1	41.3*	35.3*	21.4*
	SD	16.8	3.3	13.5	20.3	25.6
	N	5	5	5	5	4
	% Diff		-11.2	-49.2	-56.5	-70.4

*$p<0.05$

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND INTAKE OF TEST SUBSTANCE

Adverse, test substance-related reductions (compared to control) in mean food consumption were observed in rats exposed to 450 ppm and above in the diet and generally demonstrated a dose response. Differences were statistically significant over all weekly and overall intervals. Mean overall (test day 0-28) food consumption in these groups was 28-43% below control, although the largest differences were observed over the first 2 weeks at the highest two concentrations.

Adverse, test substance-related reductions (compared to control) in mean food efficiency were observed in rats exposed to 450 ppm and above in the diet and generally demonstrated a dose response. Overall mean food efficiency in these groups was 29-48% below control, although the difference was only statistically significant in the 900 ppm group. The lower mean food efficiency in all three of these groups was statistically significant over the first week of study (-11%, -92%, -320% and -405% in the 225, 450, 675 and 900 ppm groups respectively compared to control group), due to the body weight gain effects. However, mean food efficiency in these groups was not statistically significantly different from control, and exceeded that of control over many of the remaining weeks.

Overall mean daily intake in the 225, 450, 675, and 900 ppm groups was 16.23, 27.66, 42.05, and 48.49 mg/kg/day, respectively. During the first week of exposure, mean daily intake in the 900 ppm group was lower than in the 675 ppm group, due to very low food consumption in the 900 ppm group. However, mean daily intake overall and over the remaining weekly intervals did exhibit a dose response.

Table B.6.1.7-3
Mean daily food consumption (g/animal/day) by grams female rats

Diet		**0 ppm**	**225 ppm**	**450 ppm**	**675 ppm**	**900 ppm**
Days relative to start date:		**1**	**2**	**3**	**4**	**5**
0 - 7	Mean	16.9	15.1	11.2*	10.4*	7.1*
	SD	1.7	0.5	0.9	2.1	1.1
	N	5	5	5	5	5
	% Diff		-10.7	-33.9	-38.5	-58.2
7 - 14	Mean	17.2	15.6	12.3*	9.9*	8.0*
	SD	1.8	0.6	0.8	1.5	0.1
	N	5	5	5	5	5
	% Diff		-9.1	-28.1	-42	-53.2
14 - 21	Mean	17.5	15.6	12.3*	9.9*	8.0*
	SD	2.0	0.4	0.0	0.1	0.9
	N	5	5	5	5	4
	% Diff		-4	-21	-27	-31.5
21 - 28	Mean	18.5	17.4	13.0*	12.6*	12.7*
	SD	2.3	1.0	1.0	1.1	0.5
	N	5	5	5	5	4
	% Diff		-7.4	-28.1	-34.8	-43
0 - 28	Mean	17.5	16.2	12.6*	11.4*	10.0*
	SD	1.9	0.7	0.2	0.2	0.1
	N	5	5	5	5	4
	% Diff		-7.4	-28.1	-34.8	-43

*p<0.05

D. HEMATOLOGY

Changes in hematology parameters consistent with minimal to mild increased red cell turnover (hemolysis) were observed at all dietary concentrations.

Red cell mass parameters (red blood cell [RBC], hemoglobin [HGB] and hematocrit [HCT]) were decreased in female rats at 225 ppm and above (variable statistical significance; 86% to 96% of control). These changes in red cell mass parameters were associated with higher absolute reticulocyte counts (ARET), and thus were regenerative under the conditions of this study (172% to 358% of control). Changes in red cell indices consistent with the regenerative response—increased mean cell volume (MCV) and red cell distribution width (RDW), and decreased mean cell hemoglobin content (MCHC)—were also observed in these groups. These changes in hematology parameters were considered to be treatment-related and potentially adverse.

There were no other test substance-related or adverse hematology findings. Absolute eosinophil counts were decreased in female rats at 675 and 900 ppm (42% and 41% of control respectively). These changes were not associated with correlative changes in other white cell parameters, did not occur in a dose related manner and thus were likely spurious and unrelated to treatment.

Table B.6.1.7-4
Summary of haematology values for female rates

Diet			**0 ppm**	**225 ppm**	**450 ppm**	**675 ppm**	**900 ppm**
	Days relative to start date:	**Group #**	**1**	**2**	**3**	**4**	**5**
RBC ($\times 10^6$ µL)	32	Mean SD N % Diff	8.15 0.21 5	7.60* 0.20 5 -6.8	7.46* 0.24 4 -8.5	6.99* 0.39 4 -14.3	7.19* 0.26 3 -11.8
HGB (g/dL)	32	Mean SD N % Diff	15.2 0.5 5	13.9* 0.3 5 -8.4	13.9* 0.8 4 -8.3	13.2* 0.5 4 -13.4	14.2 0.6 3 -6.7
HCT (%)	32	Mean SD N % Diff	44.9 1.3 5	41.7* 1.1 5 -7.1	42.0* 1.5 4 -6.5	40.5* 2.4 4 -10	43.2 1.6 3 -3.9
MCV ($\times 10^6$ µL)	32	Mean SD N % Diff	55.1 1.2 5	55.0 2.3 5 -0.2	56.3 1.3 4 2.2	58.0 1.2 4 5.1	60.2* 3.1 3 9.1
MCH (pg)	32	Mean SD N % Diff	18.6 0.4 5	18.3 0.7 5 -1.5	18.7 0.8 4 0.3	18.8 0.5 4 1	19.7 1.0 3 6
MCHC (g/dL)	32	Mean SD N % Diff	33.8 0.1 5	33.4 0.5 5 -1.3	33.1 0.8 4 -2	32.5* 0.7 4 -3.8	32.8* 0.4 3 -3.1
RDW (%)	32	Mean SD N % Diff	10.5 0.1 5	11.4* 0.2 5 9.2	12.8* 0.3 4 22.1	14.7* 1.4 4 40.8	13.8* 1.5 3 32.2
PLT ($\times 10^3$ µL)	32	Mean SD N % Diff	1178 130 5	1171 207 5 -0.6	1174 49 4 -0.4	1276 259 4 8.3	1404 109 3 19.2
WBC ($\times 10^3$ µL)	32	Mean SD N % Diff	10.34 3.63 5	9.42 1.62 5 -8.9	10.74 1.92 4 3.9	8.18 0.94 4 -20.9	6.38 2.36 3 -38.3
ARET ($\times 10^3$ µL)	32	Mean SD N % Diff	177.7 19.4 5	306.9* 34.3 5 72.7	363.6* 55.2 4 104.6	636.2* 107.3 4 257.9	400.7* 85.8 3 125.5

*$p<0.05$

E. ORGAN WEIGHT DATA

A test substance-related increase in spleen weight parameters was observed in female rats at all dietary concentrations (225 ppm and above), as compared to control values.

Mean relative (% body weight) spleen weights were increased 23%, 30%, 38%, and 21% in the 225, 450, 675, and 900 ppm dietary concentration groups, as compared to control values. These increases were statistically significant in all but the 900 ppm dose group and reflected the opposing trends of increasing spleen weights and decreasing terminal body weights.

Mean absolute and mean relative (% brain weight) spleen weights were also increased at all but the highest concentration where general toxicity appeared to mute the splenic effect. However, these differences were not statistically significant.

The spleen weight increases correlated with microscopic findings (increased hematopoiesis, increased pigment, and dilated sinusoids) at 450 ppm and above.

Table B.6.1.7-5
Mean absolute and relative spleen weights in female rats

Group:	**1**	**2**	**3**	**4**	**5**
Dietary Concentration (ppm):	**0**	**225**	**450**	**675**	**900**
Female (number of rats)	(5)	(5)	(5)	(5)	(4)
mean terminal body wt. (grams)	253.8	247.7	218.0	212.1#	198.5#
% difference from control	-	-2%	-14%	-16%	-22%
Spleen					
absolute wt. (grams)	0.524	0.626	0.588	0.603	0.496
spleen wt./brain wt. x 100	28.776	32.812	31.400	32.848	26.829
spleen wt./body wt. x 100	0.206	0.253#	0.268#	0.285#	0.250
% difference from control*	-	+23%	+30%	+38%	+21%

wt. = weight

= statistically significant (Dunnett 2-sided $p<0.05$; parametric), compared to Group 1 (control) value.

Underlined values were interpreted to be test substance-related increases, compared to Group 1 (control).

* = % difference from control calculated from mean relative to body weight data.

Several mean organ weight parameters were statistically significantly increased or decreased as a result of general body weight loss in rats fed diets containing 450 ppm or more of the test substance. These included organs that lose weight in relative proportion to general body weight (heart, kidneys, and liver) and, therefore, had no statistically significant differences in their mean % body weight values. It also included organs that resist weight loss (brain) and therefore had no differences in their mean absolute weight values. In addition, the adrenals and ovaries, which had statistically significant decreases in mean absolute and/or mean % brain weight values, had no significant effect on their % body weight values. There was no microscopic correlate for those tissues that were examined microscopically (liver and kidneys) except for the hepatocellular atrophy observed in the decedent group 5 female (rat #554).

All other individual and mean organ weight differences were considered to be spurious and unrelated to test substance administration.

F. MICROSCOPIC FINDINGS

In surviving rats, test substance-related microscopic findings were only observed in the spleen (450 ppm and above). Splenic effects were consistent with increased red cell turnover and included increased hematopoiesis, increased pigment, and sinusoid dilatation.

Table B.6.1.7-6
Incidence and severity of test substance-related microscopic findings in the spleen and bone marrow of surviving female rats

Group:	**1**	**2**	**3**	**4**	**5**
Dietary Concentration (ppm):	**0**	**225**	**450**	**675**	**900**
Female (number of rats)	(5)	(5)	(5)	(5)	(4)
Spleen: Hematopoiesis, increased	0	0	5	5	3
minimal	-	-	4	0	2
mild	-	-	1	5	1
Spleen: Pigment, increased	0	0	5	5	4
mild	-	-	5	5	4
Spleen: Dilated sinusoids (i.e. congestion)	0	0	5	5	4
minimal	-	-	2	0	0
mild	-	-	3	5	1
moderate	-	-	0	0	3
Bone Marrow: Atrophy/depletion	0	0	0	0	1
minimal	-	-	-	-	1

Note: Underlined values were interpreted to be test substance-related increases.

Daily dietary exposure of female rats to 225, 450, 675, and 900 ppm of the test substance, for approximately 28 days, resulted in one death (900 ppm), increased spleen weights (225 ppm and above), and microscopic effects in the spleen (450 ppm and above) and bone marrow (900 ppm). Splenic effects, including increased hematopoiesis, increased pigment, and dilated sinusoids, were consistent with increased red cell turnover. Bone marrow atrophy/depletion in one 900 ppm rat was indicative of hematopoietic toxicity.

Conclusions proposed by the applicant (2015):
Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for Indoxacarb 150 g/L EC was less than 225 ppm (less than 16.23 mg/kg body weight/day) in female rats, based on hematology and microscopic pathology evidence of hematotoxicity at all dietary concentrations.

RMS FR assessment (2016):
Adverse effects related to haemolytic anemia were observed from the low dose level of 225 ppm (equivalent to 16.23 mg/kg bw/d) of Indoxacarb 150 g/LG EC in this 28-day study. This study investigated only some parameters and was carried out in female rats only.

90-day study with the formulation Indoxacarb 150 g/L EC:

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.1.7/02

Report: [redacted] (2012b); Indoxacarb (DPX-KN128) 150 g/L EC (pure active substance indoxacarb): Subchronic toxicity 90-day feeding study in rats

DuPont Report No.: DuPont-33600

Guidelines: OPPTS 870.3100 (1998), OECD 408 (1998), EEC Method B.26 (2008), MAFF 12 Nousan 8147 (2000) **Deviations:** Only female rats were tested since they are the most sensitive gender and species. No neurobehavioral evaluations (FOB/MA) were conducted.

Testing Facility: [redacted]

Testing Facility Report No.: DuPont 33600

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Indoxacarb 150 g/L EC
	Lot/Batch #:	KN128-311 / JUL10BL024
	Purity:	15.1% (143.0 g/L) indoxacarb active ingredient, 84.9% inert ingredients
	Description:	Light yellow/amber liquid
	CAS #	None for the formulation 173584-44-6 for active substance indoxacarb
	Stability of test compound:	Analyses confirmed that test material was stable in feed for at least 22 days at room temperature or refrigerated, was homogeneously mixed, and was present in the feed at targeted concentrations. Batches were prepared at weekly or biweekly intervals.
2.	Vehicle and/or negative control:	Acetone and untreated diet
3.	Test animals	
	Species:	Rat
	Strain:	Crl:CD(SD)
	Age at initial dosing:	Approximately 7 weeks old
	Weight at initial dosing:	173.4–209.9 g
	Source:	[redacted]
	Acclimation period:	8 days
	Diet (or dosing solution, if a gavage study):	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed in pairs in solid-bottom caging with bedding mixed with appropriate species-specific enrichment.
4.	Environmental conditions	
	Temperature:	20–26°C
	Humidity:	30–70%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   27-January-2012 to 02-May-2012

2. Animal assignment and treatment

   Four groups of 10 females/concentration were administered Indoxacarb 150 g/L EC in feed daily for at least 90 days. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (NRC 2011).
   The dietary concentrations selected for this study were based on the results of a range-finding study (DuPont-33601).
   Based on the results of the pilot study, female rats in the 90-day study were administered diet containing 0, 62.5, 125, or 250 ppm, corresponding to mean daily intakes of 3.82, 7.53 and 15.23 mg/kg bw/day.

3. Diet preparation and analysis

The test substance, Indoxacarb 150g/L EC (dissolved in acetone) was added to the rodent diet and thoroughly mixed for a period of time that was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. All diets were prepared weekly or biweekly, and refrigerated until used. The stability, homogeneity, and concentration of Indoxacarb 150 g/L EC in the dietary mixtures were checked by analysis using HPLC with UV detection at the beginning of the study, and concentration was verified during the first month and near the middle and end of study. The test substance was at target concentrations (±17% of nominal), homogeneously mixed (RSD ≤7%) throughout the feed, and was stable (±8% of nominal) for up to 22 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Significance was judged at $p < 0.05$.

Table B.6.1.7-7
Statistics: 90-day feeding study in female rats

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Clinical pathology[a] Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normalcy	One-way analysis of variance followed with Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with those bilirubin data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with those data.

C. METHODS

1. Observations

Animals were observed at least twice daily for mortality and morbidity and for signs of abnormal behaviour and appearance and daily for signs of acute systemic toxicity. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance, and subjected to detailed clinical observations.

2. Body weights

All animals were weighed once per week. All animals were weighed on the day of sacrifice.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, coagulation, and urinalysis)

Blood and urine samples were collected from all animals, 95 and 96 days after initiation of the study. Animals were fasted approximately 15 hours prior to sample collection. At sacrifice blood, bone marrow, and urine were collected. Evaluation of haematology, clinical chemistry, coagulation, bone

marrow smear, and urinalysis parameters were performed for all animals. Bone marrow smears were prepared at the final sacrifice from all animals, but analysis was not necessary to support experimental findings.

6. Sacrifice and pathology

At termination (test Days 95 and 96), animals were sacrificed by exsanguination while under isoflurane anaesthesia. Gross examinations were performed on all animals. Organs that were weighed are listed in the table belowTable B.6.1.7-. Group mean values and organ weight ratios (% body weight and % brain weight) were calculated. Tissues collected from animals receiving the highest concentration (250 ppm) and control (0 ppm) were processed to slides and evaluated microscopically. Gross lesions and suspected target tissues (spleen, bone marrow, and liver) from rats in the low and intermediate dose groups (Groups 2 and 3) were also processed and examined.

Table B.6.1.7-8
90-Day feeding study in female rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Brain	X	X
Spleen	X	X
Heart	X	X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Skin		X
Trachea		X
Nose		X
Larynx/pharynx		X
Thymus	X	X
Mesenteric lymph node		X
Mandibular lymph node		X
Bone marrow		X
Peyer's patches		X
Thyroid gland		X
Parathyroid glands		X
Eyes		X
Ovaries (including oviducts)	X	X
Uterus (including cervix)	X	X
Vagina		X
Mammary glands (females)		X
Stomach		X
Pituitary		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Optic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs of toxicity were observed at any dietary concentration in either males or females.

2. Mortality

Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

At test Day 91 body weights were 9 and 6% lower than the controls in the 250 and 125 ppm groups, respectively. The overall body weight gains were 18 and 14% lower than the controls in the 250 and 125 ppm groups, respectively. None of these differences were statistically significant. They were, however, considered adverse based on the magnitude of difference in body weight gain, and the correlation with lower food consumption and food efficiency. Mean body weight (250 ppm) and body weight gain (250 and 125 ppm) were statistically significantly lower than the control over one or more weekly intervals. No test substance-related effects on body weight or body weight gain were observed in the 62.5 ppm group.

Table B.6.1.7-9
90-Day feeding study in female rats: Body weights (g)

Day	Indoxacarb 150 g/L EC			
	0 ppm	**62.5 ppm**	**125 ppm**	**250 ppm**
Day 0	186.5	185.9	186.9	184.2
Day 91	340.1	334.3	319.1	310.2

Table B.6.1.7-10
90-Day feeding study in female rats: Body weight gain (g)

Parameter	Indoxacarb 150 g/L EC			
	0 ppm	**62.5 ppm**	**125 ppm**	**250 ppm**
Overall body weight gain, Day 0–91	153.6	148.4	132.2	126.1

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Female rats administered the 250 and 125 ppm diets had statistically significantly lower food consumption over test Days 0-91, compared to controls (9% lower for 250 ppm and 7% lower for 125 ppm). Mean overall food efficiency was 9 and 8% below control, respectively, in the 250 and 125 ppm groups (neither statistically significant). The differences in mean weekly food efficiency were only statistically significant over one interval in the 250 ppm group and over no intervals in the 125 ppm group, and weekly food efficiency exceeded that of control over some weekly intervals (not statistically significant). Based on the magnitude of difference and the correlation with lower body weight gain and food consumption, however, these differences were considered mild but adverse. No test substance-related effects on food consumption or food efficiency were observed in the 62.5 ppm group.

The mean daily intakes for female rats were 3.82, 7.53, and 15.23 mg/kg bw/day for the 62.5, 125, and 250 ppm groups, respectively.

Table B.6.1.7-6-11
90-Day feeding study in female rats: Food consumption (g/animal/day)

Parameter	Indoxacarb 150 g/L EC			
	0 ppm	62.5 ppm	125 ppm	250 ppm
Food consumption, Day 0–91	18.0	17.7	16.8*	16.3*

* Significantly different from control by the Dunnett non-parametric 2 sided criteria, p <0.05.

Table B.6.1.7-12
90-Day feeding study in female rats: Food efficiency (average weight gain/average food consumed)

Parameter	Indoxacarb 150 g/L EC			
	0 ppm	62.5 ppm	125 ppm	250 ppm
Food efficiency, Day 0–91	0.094	0.092	0.086	0.085

D. OPHTHALMOLOGICAL EXAMINATIONS

No test substance-related ophthalmological observations were observed at any dietary concentration in female rats.

E. CLINICAL PATHOLOGY

1. Haematology

Minimal decreases in red cell mass parameters (red blood cells [RBC], haemoglobin [HGB], and haematocrit [HCT]), with an associated minimal increase in absolute reticulocyte count (ARET), were present in the 125 and 250 ppm groups (statistically significant at 250 ppm; only RBC statistically significant at 125 ppm). These haematology changes at 125 and 250 ppm were considered test substance-related. The haematology changes were associated with secondary physiological responses in the spleen at these dietary concentrations. There were no other statistically significant or treatment-related haematology findings.

Table B.6.1.7-13
90-Day feeding study in female rats: Haematology findings at week 13

Parameter	Indoxacarb 150 g/L EC			
	0 ppm	62.5 ppm	125 ppm	250 ppm
RBC (10^6/µL) % difference to control	8.33	8.36	7.93* -4.8%	7.87* -5.6%
HGB (g/dL) % difference to control	15.2	15.1	14.5 -4.5%	14.2* -6.7%
HCT (%) % difference to control	45.9	45.8	43.6 -5.1%	43.5* -5.3%
ARET (10^3/µL) % difference to control	150.7	141.1	178.7 +18.6%	216.1* +43.4%

* Significantly different from control by the Dunnett 2 sided criteria, p <0.05.

2. Clinical chemistry

There were no statistically significant or biologically significant changes in clinical chemistry parameters in female rats.

3. Coagulation

There were no statistically significant or biologically significant changes in coagulation parameters in female rats.

4. Urinalysis

There were no adverse changes in urine parameters in female rats.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

No test substance-related changes in mean absolute organ weights or relative organ weights (relative to final body weight or to brain weight) were apparent at any dietary concentration.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy. Test substance-related microscopic findings were observed in the spleen in 125 and 250 ppm females and consisted of a minimal increase in haemosiderin pigment, which correlated with the haematology findings of slightly decreased red cell mass parameters at the same dietary concentrations. At stated in the study report, the pigment was within the reticuloendothelial cells (i.e. fixed macrophages) of the red pulp and was golden brown in color. The pigment was consistent with hemosiderin. All other microscopic findings were consistent with normal background lesions in rats of this age and strain.

Table B.6.1.7-6-14
90-Day feeding study in female rats: Incidences of microscopic effects

Indoxacarb 150 g/L EC (ppm):	**0**	**62.5**	**125**	**250**
Number of rats/group:	**10**	**10**	**10**	**10**
Spleen				
Pigment, increased, minimal	0	0	4*	6*

* Interpreted to be test substance-related increases.

Conclusions proposed by the applicant (2015):
The NOAEL was 62.5 ppm Indoxacarb 150 g/L EC (3.82 mg/kg bw/day) for female rats. This NOAEL was based on mild reductions in body weight and nutritional parameters at 125 ppm Indoxacarb 150 g/L EC (7.53 mg/kg bw/day) and above.
Taking into account the effects observed in the pilot study and the definitive 90-day study with Indoxacarb 150 g/L EC, it should be classified as Category 2 for Specific Target Organ Toxicity following Repeated Exposure (STOT-RE) (H373) according to the provisions of Regulation (EC) No. 1272/2008.

RMS FR assessment (2016):
The NOAEL of this study, performed in female rats only, is set at 62.5 ppm (3.82 mg/kg bw/d) of Indoxacarb 150 g/L EC, based on decreased body weights, body weight gains and food consumption, as well as findings related to haemolytic anemia (decreased red blood cell parameters and increased reticulocytes, as well as haemosiderin deposits in the spleen of 40% of the tested rats) at the dose level of 125 ppm (7.53 mg/kg bw/d) of Indoxacarb 150 g/L EC.

Therefore, based on these findings, Indoxacarb 150 g/L EC should be classified as Category 1 for Specific Target Organ Toxicity following Repeated Exposure (STOT-RE) (H372) according to the provisions of Regulation (EC) No. 1272/2008. This classification is also in line with the content of indoxacarb (15%) in the formulation. Indeed, according to Regulation (EC) No. 1272/2008, a mixture shall be classified STOT RE1 if it contains ≥ 10% of an ingredient classified as STOT RE1.
It should be noted that indoxacarb has been classified by ECHA as Category 1 for Specific Target Organ Toxicity following Repeated Exposure taking into account not only haematological effects, but also neurotoxic and heart effects (see Volume 3B6-CA). As the 28- and 90-day studies performed on the preparation did not cover parameters related to these last effects, the proposal of the applicant can anyway not be agreed.

B.6.1.8. Supplementary studies for combinations of plant protection products

No supplementary studies are required to satisfy the data requirements for registration of Indoxacarb 150 g/L EC.

B.6.2. DERMAL ABSORPTION

The dermal absorption of indoxacarb from Indoxacarb 150 g/L EC was investigated *in vivo* in the rat (DuPont-38097) and *in vitro* using both rat and human skin (DuPont-38096). Tests were performed with the undiluted formulation and with a 0.4 g/L dilution, plus an additional 0.04 g/L dilution *in vitro* only. Species difference ratios in absorption were determined using the data from the study report DuPont-38096 since investigations were conducted using both human and rat skin in the same study under identical test conditions (same test material/formulation/vehicle/concentrations and similar exposure time and swabbing technique).

The results are summarised in the following table. *In vitro* test results reflect determinations made 18 hours after a 6-hour dose period. *In vivo* test results reflect determinations made 66 or 138 hours after a 6-hour dose period for the concentrate and diluted formulations respectively.

Table B.6.2-1
Dermal absorption of indoxacarb in different test systems

Study	Formulation concentrate		0.4 g/L dilution		0.04 g/L dilution	
	Maximal flux (μg/cm²/h)	**Absorbed dose (%)**	**Maximal flux (μg/cm²/h)**	**Absorbed dose (%)**	**Maximal flux (μg/cm²/h)**	**Absorbed dose (%)**
In vitro - rat skin	16	19.35	0.17	54.7	0.016	46.8
In vitro - human skin	0.52	10.01	0.017	43.3	0.0013	36.6
In vivo - rat	-	3.35	-	22.4	-	-

Using the triple pack approach, human *in vivo* absorption values are calculated using the following formula:

In vivo human absorption = [(*in vivo* rat absorption) x (*in vitro* human absorption)]/(*in vitro* rat absorption).

Indoxacarb 150 g/L concentrate: human *in vivo* = (3.35 x 10.01)/19.35 = 1.7%, rounded to 2%

Indoxacarb 0.4 g/L dilution: human *in vivo* = (22.4 x 43.3)/54.7 = 17.7%, rounded to 18%

The concentrations of the diluted product tested *in vitro* generally cover the range of recommended in-use concentrations (0.0375 to 0.375 g a.s./L). Since, according to the *in vitro* human/rat skin study, percent of dose absorbed for a given species is relatively consistent once the product is diluted (0.04 g/L and 0.4 g/L) no adjustment of dermal absorption is needed for different in-use spray concentrations.

It is to be noted that the applicant proposed to use maximal flux to derive the species ratio. Based on the following statement: "*Since DuPont-38096 investigated both rat and human skin absorption in the same study under identical test conditions it is valid to use maximal flux to derive the species ratio. There was not a significant difference in the length of the linear phase between rat and human skin. Furthermore, the ratio between rat and human skin for the total amount that penetrated into the receptor fluid through the 24 hour collection period was similar to the ratio between rat and human skin for maximal flux*". Using this approach, the dermal absorption values are 0.1% and 2.24% for the undiluted and 0.4 g/L dilution respectively.

It is to be noted that an *in vitro* study using only human skin (DuPont-19442) was firstly submitted but was not further supported by the applicant. This *in vitro* human skin study was performed using a diluted formulation not representative of the in-use dilutions. Moreover, the detail of radioactivity recovered in each tape strip was not available in the study report. For these reasons, and taking into account that two acceptable and well-performed studies are available for Indoxacarb 150 g/L EC, the RMS agrees not to take into account this further study.

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.3/03
Report: [redacted] (2014b); Indoxacarb (DPX-KN128) 150 g/L EC: *In vivo* dermal absorption of indoxacarb in the rat

DuPont Report No.: DuPont-38097

Guidelines: OECD 427 (2004), OECD 28 (2004), EFSA PPR Journal 2012;10(4):2665 **Deviations:** None

Testing Facility: [redacted]

Testing Facility Report No.: DuPont-38097

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Indoxacarb 150 g/L EC
	Lot/Batch #:	KN128-434
	Purity:	150 g a.s./L, 15.8% w/w nominal 153.5 g a.s./L, 16.2% w/w analysed
	Description:	Liquid
	CAS #	None for the formulation. Indoxacarb: 173584-44-6
	Stability of test compound:	The test material was stable under the conditions of this study.
2.	Radiolabel test material:	[^{14}C]indoxacarb
	Lot/Batch #:	[Indanone-1-^{14}C]indoxacarb, lot no. 1643850
	Radiochemical purity:	98.8%
	Specific activity:	49.50 µCi/mg
	Description:	Solid
	Stability of test compound:	The test material was stable under the conditions of this study.
3.	Indoxacarb 150 g/L EC formulation blank:	The blank formulation, which was devoid of indoxacarb, was blended with radiolabeled indoxacarb to produce the aqueous spray formulation. The blank formulation was stored at room temperature.
4.	Vehicle and/or positive control:	Water for the aqueous dilution
5.	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)
	Gender	Male
	Age at dosing:	Approximately 7–8 weeks old
	Weight at dosing:	181–219 g
	Source:	[redacted]
	Acclimation period:	Six days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed in all-glass metabolism units
6.	Environmental conditions	
	Temperature:	20–26°C
	Humidity:	30–70%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion:

   19-August-2014 to 09-October-2014

2. Animal assignment and treatment

   On the day prior to dermal dosing, the back and shoulders of each animal were clipped free of hair and the clipped area washed with deionized water. Following clipping and washing, 3 silicone O-rings (one stacked upon the other) with an internal area of approximately 10.5 cm², were glued to the clipped area on the back using Instant Krazy Glue® Gel. The O-rings were then covered with Coban™ body wrap (3M Company, St. Paul, Minnesota, USA) to prevent contamination of the dose site.
   Doses applied and target parameters are summarised in the following table.

Table B.6.2-2
Study design: Summary of doses applied and target parameters for the *in vivo* assessment of dermal absorption of indoxacarb in the rat

Group	Dose concentration	Skin dose level	Number of animals[a]	µCi/rat
A	150 g a.s./L[b] (undiluted concentrate)	1500 µg/cm^2	12	10.5
B	0.4 g a.s./L[c] (aqueous dilution)	4 µg/cm^2	12	2

[a] Based on four rats at three post-exposure collection time points

[b] Rats in group A were exposed to a single application of the undiluted formulation concentrate.

[c] Rats in group B were exposed to a single application of an aqueous dilution of the formulation.

Three sacrifice time intervals for Group A and Group B treatment groups were included in this study:
Interval I: Four animals were sacrificed 24 hours after dermal application (18 hours post-exposure).
Interval II: Four animals were sacrificed 72 hours after dermal application (66 hours post-exposure).
Interval III: Four animals were sacrificed 144 hours after dermal application (138 hours post-exposure).

3. Dose formulation and analysis

[Indanone-1-^{14}C]indoxacarb was dissolved into the EC formulation blank to produce the undiluted concentrate (150 g a.s./L). The 0.4 g a.s./L aqueous dilution was prepared by dissolving radiolabeled [Indanone-1-^{14}C]indoxacarb in EC formulation blank, followed by the addition of deionized water.
The homogeneity and amount of radiolabeled indoxacarb (µCi/g) in each formulated dose was determined by subjecting aliquots of the prepared dose to radioanalysis by liquid scintillation counting (LSC). The concentration of indoxacarb in each dose formulation was based on weights and/or volumes of formulation ingredients. The results of homogeneity and concentration analyses were used to calculate the specific activity of radiolabeled indoxacarb (µCi/mg) for the formulated doses.
The radiochemical purity of the neat radiolabeled indoxacarb and the stability of radiolabeled indoxacarb in the prepared dose formulations were determined by HPLC with an in-line radiochemical detection system.

4. Dosing

The formulated products were applied to a 10.5 cm^2 shaved area on the dorso-lumbar region of each rat at a rate of 10 µL/cm^2. The dose site was protected with a non-occlusive, rigid mesh covering, O-ring spacers and Coban™ body wrap. The applied formulation remained in contact with the skin for 6 hours. After 6 hours, the skin surface of all rats was washed using a mild soap solution (0.2% Ivory® soap solution).

5. In-life sample collection

Urine and faeces were collected during the 0–6 hour exposure period, at 6–12 hours, 12–24 hours, and every 24 hours thereafter until sacrifice. The 6-hour skin wash, body wrap, and protective covering were collected for analysis.

6. Sacrifice

Animals were anesthetised using isoflurane and euthanised by exsanguination. The application skin site was excised and tape-stripped to remove the *stratum corneum*. Final skin washes, O-rings, mesh cover, bandages, cage washes, residual feed, skins from the application site, tape strips, skins from the non-dosed area, blood (whole blood, plasma and red blood cells), and remaining carcass were analysed.

7. Sample analysis

Urine, skin washes, cage washes, and blood plasma were assayed directly by LSC. The protected mesh cover, body wrap, O-ring spacers, and tape strips were extracted using acetonitrile and analysed directly

by LSC. Faeces, residual feed, and carcasses were homogenised in water and combusted prior to LSC. Skin samples and wash sponges were digested in Solvable™ and analysed directly by LSC.

8. Statistics

Group data are represented as mean ± SD.

II. RESULTS AND DISCUSSION

A. RADIOCHEMICAL PURITY, CONCENTRATION AND STORAGE STABILITY

The purity of the neat radiolabeled indoxacarb was 96.1%. Analyses confirmed that indoxacarb was present in the dosing formulations at the appropriate concentrations and was appropriately radiolabeled. The radiochemical purity of the [Indanone-1-^{14}C]indoxacarb in the dose formulations ranged from 95.0% to 94.3% and was considered adequate for testing.

B. TREATMENT GROUP A

Treatment group A was exposed to Indoxacarb 150 g/L EC concentrate. The majority of the applied radioactivity was recovered in the skin washes and bandages. Low levels of radioactivity were recovered in the excreta, the application site (after washing), surrounding skin area, and carcass.

Table B.6.2-3
Absorption of radiolabeled indoxacarb by rats exposed to Indoxacarb 150 g/L EC concentrate
(Treatment group A)

Absorption:	**Percent of administered radioactivity (%)**		
	18 h post-exposure	**66 h post-exposure**	**138 h post-exposure**
Skin washes	82.960	81.181	83.836
Tape strips 1-10	10.600	9.170	6.790
Protective device[a]	4.634	7.150	7.022
Absorbed dose:			
Urine	0.466	0.704	0.896
Faeces	0.145	0.363	0.429
Whole blood	0.004	0.003	0.002
Plasma	0.003	0.002	0.001
Red blood cells	0.003	0.002	0.001
Carcass	0.187	0.218	0.110
Cage wash	0.130	0.318	0.221
Residual feed	0.003	0.020	0.022
(Stripped) skin	0.105	0.091	0.034
Non-dosed skin	0.015	0.013	0.006
Tape-strips 3-10	1.303	0.784	0.405
Total absorbed dose[b]:	2.36 ± 0.73	2.52 ± 0.83	**2.11** ± 0.42
Total absorbed dose corrected for high variability:	**3.09**	**3.35**	Correction not necessary
Total recovered (material balance):	99.2	99.2	99.4
Total absorbed dose corrected for low recovery:	Correction not necessary	Correction not necessary	Correction not necessary

[a] Includes rigid mesh covering, O-ring spacers, Coban™ body wrap, and forceps rinse

[b] including tape strips 3 to 10 as the majority of absorption did not occur within half of the duration of the sampling periods

Table B.6.2-4
Percent distribution of radiolabeled indoxacarb in tape strips (*stratum corneum*) from the skin of rats exposed to Indoxacarb 150 g/L EC concentrate
(Treatment group A)

	18 h post-exposure		66 h post-exposure		138 h post-exposure	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Tape strip 1	6.595	0.294	7.303	1.296	5.658	2.297
Tape strip 2	2.732	3.102	1.087	0.292	0.728	0.484
Tape strip 3	0.764	0.560	0.341	0.176	0.273	0.175
Tape strip 4	0.272	0.129	0.245	0.136	0.057	0.012
Tape strip 5	0.139	0.110	0.111	0.060	0.036	0.021
Tape strip 6	0.050	0.031	0.037	0.014	0.015	0.010
Tape strip 7	0.040	0.030	0.026	0.005	0.014	0.015
Tape strip 8	0.010	0.006	0.012	0.004	0.005	0.002
Tape strip 9	0.012	0.011	0.008	0.001	0.003	N.A.
Tape strip 10	0.016	0.025	0.004	0.002	0.002	N.A.
Total tape strips 3 to 10	1.303		0.784		0.405	

C. TREATMENT GROUP B

Treatment group B was exposed to a 0.4 g a.s./L aqueous solution of Indoxacarb 150 g/L EC (equivalent to approximately 375-fold dilution of the field application solution concentration). The majority of the applied radioactivity was recovered in the skin washes and bandages. Low levels of radioactivity were recovered in the excreta, the application site (after washing), surrounding skin area, and carcass.

Table B.6.2-5
Absorption of radiolabeled indoxacarb by rats exposed to Indoxacarb 150 g/L EC aqueous dilution (Treatment group B)

Absorption:	Percent of administered radioactivity (%)		
	18 h post-exposure	**66 h post-exposure**	**138 h post-exposure**
Skin washes	63.942	56.198	53.585
Tape strips	9.51	14.8	10.4
Protective device[a]	7.322	7.387	8.302
Absorbed dose:			
Urine	2.226	3.847	6.920
Faeces	0.641	1.533	3.216
Whole blood	0.027	0.026	0.035
Plasma	0.015	0.013	0.015
Red blood cells	0.030	0.022	0.020
Carcass	1.252	0.964	0.891
Cagewash	0.467	1.083	1.797
Residual feed	<LOQ	<LOQ	<LOQ
(Stripped) skin	1.007	0.540	0.430
Non-dosed skin	0.017	0.016	0.017
Tape strips 3-10	3.211	2.383	1.104
Total absorbed dose[b]:	8.88 ± 1.68	10.4 ± 3.1	14.4 ± 5.5
Total absorbed dose corrected for high variability:	Correction not necessary	13.5	19.9
Total recovered (material balance):	**86.4**	**86.4**	**85.5**
Total absorbed dose corrected for low recovery:	**10.3**	**15.1**	**22.4**

[a] Includes rigid mesh covering, O-ring spacers, Coban™ body wrap, and forceps rinse

[b] including tape strips 3 to 10 as the majority of absorption did not occur within half of the duration of the sampling periods

Table B.6.2-6
Percent distribution of radiolabeled indoxacarb in tape strips (*stratum corneum*) from the skin of rats exposed to 0.4 g indoxacarb /L EC aqueous dilution (Treatment group B)

	18 h post-exposure		**66 h post-exposure**		**138 h post-exposure**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Tape strip 1	3.197	0.170	7.615	0.879	6.876	4.277
Tape strip 2	3.111	0.737	4.861	1.511	2.516	1.159
Tape strip 3	2.040	1.005	1.542	1.194	0.739	0.433
Tape strip 4	0.785	0.511	0.551	0.509	0.206	0.194
Tape strip 5	0.269	0.223	0.126	0.150	0.077	0.067
Tape strip 6	0.072	0.079	0.075	0.084	0.062	NA
Tape strip 7	0.019	0.015	0.030	0.032	0.020	NA
Tape strip 8	0.009	0.005	0.025	NA	NA	NA
Tape strip 9	0.009	0.003	0.024	NA	NA	NA
Tape strip 10	0.008	NA	0.010	NA	NA	NA
Total tape strips 3 to 10	3.211		2.383		1.104	

III. CONCLUSIONS

The *in vivo* dermal absorption of [^{14}C]indoxacarb in the Indoxacarb 150 g/L EC formulation, at two concentration levels undiluted formulation concentrate and 0.4 g a.s./L aqueous solution (approximately 375-fold dilution of the field application solution concentration) in male rats was investigated. Maximum absorption was higher for the aqueous dilution compared to the neat formulation. The values for percent absorbed dose listed below were corrected as needed if the total recovery was less than 95% or if the standard deviation was greater than 25% of the mean absorption value.

Dose formulation	Total absorbable dose at end of 6-hour exposure (18 h post exposure)	Total absorbable dose following 66 hour recovery/collection period	Maximum total absorbable dose following 138 hour recovery/collection period
Undiluted concentrate 150 g/L	3.09%	3.35%	2.11%
Aqueous solution 0.4 g/L	10.3%	15.1%	22.4%

Previous evaluation:	**Submitted for the purpose of renewal**

CP 7.3/02

Report: Mingoia, R.T. (2014a); Indoxacarb (DPX-KN128) 150 g/L EC: *In vitro* percutaneous absorption of indoxacarb in rat and human skin

DuPont Report No.: DuPont-38096

Guidelines: OECD 428 (2004), OECD 28 (2004) **Deviations:** None

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: DuPont-38096

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

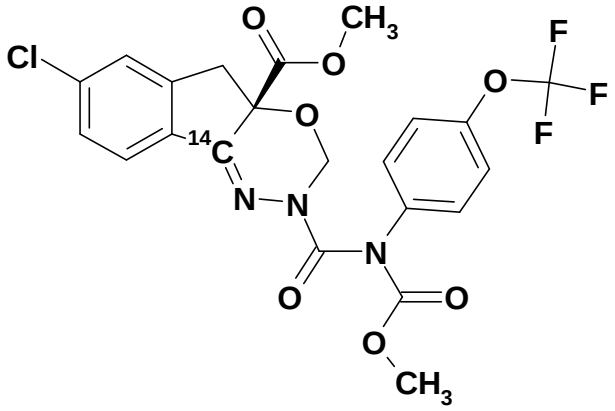
1.	Test material:	Indoxacarb 150 g/L EC
	Lot/Batch #:	KN128-434
	Purity:	150 g a.s./L, 15.8% w/w nominal 153.5 g a.s./L, 16.2% w/w analysed
	Description:	Clear, light yellow liquid
	CAS #	None for the formulation 173584-44-6 for active substance indoxacarb
	Stability of test compound:	The test material was stable under the conditions of this study.
2.	Radiolabel test material:	[^{14}C]indoxacarb technical
	Structure ^{14}C: Denotes position of radiolabel	
	Lot/Batch #:	[Indanone-1-^{14}C]indoxacarb lot #: 1643850
	Radiochemical purity:	98.8% (10/25/2012); 97.0% (11/02/2012)
	Specific activity:	49.50 μCi/mg
	Description:	Solid
	Stability of test compound:	The test material was stable under the conditions of this study.
3.	Indoxacarb 150 g/L EC formulation blank:	The blank formulation, which was devoid of indoxacarb, was blended with radiolabelled indoxacarb to produce the aqueous spray dilutions. The blank formulation was stored at room temperature.
4.	Rat skin:	Skin used was from male rats of the Sprague-Dawley strain, Crl:CD®(SD), approximately 6–8 weeks of age. Rats were sacrificed by carbon dioxide asphyxiation while under isoflurane anesthesia, and the fur from the dorsal region was carefully shaved using clippers. Any animals showing obvious abrasion within the region of the test skin area were considered unsuitable and discarded. The shaved area was excised, held briefly on wet ice, and then frozen at approximately -20°C until processed.
5.	Human skin:	Samples of human skin derived from the abdomen from National Disease Research Interchange (NDRI, Philadelphia, Pennsylvania, U.S.A.) were stored frozen at approximately -20°C until prepared for use.
6.	Test substance concentrations:	See Table B.6.2-7

Table B.6.2-7
Summary of the formulation, target concentration, and skin dose

Formulation	Target concentration	Target skin dose
Indoxacarb 150 g/L EC (undiluted concentrate)	150 g a.s./L	1500 μg a.s./cm^2
Indoxacarb 150 g/L EC (aqueous dilution)	0.4 g a.s./L	4 μg a.s./cm^2
Indoxacarb 150 g/L EC (aqueous dilution)	0.04 g a.s./L	0.4 μg a.s./cm^2

B. STUDY DESIGN AND METHODS

1. Experimental start/completion dates

22-July-2014 to 16-September-2014

2. Dermal penetration and absorption assay

The dermal penetration and absorption of [^{14}C]indoxacarb active substance from the Indoxacarb 150 g/L EC formulation was measured *in vitro* through rat and human skin. Frozen samples of rat and human skin were thawed, and full thickness skin was dermatomed to approximately 350 μm. Each skin membrane was mounted over the receptor chamber of a glass *in vitro* diffusion cell. The receptor chamber, containing 50% (v/v) ethanol in deionized water as the receptor fluid, was maintained at 32°C. During the exposure phase, continuous magnetic stirring of the receptor chamber was maintained to facilitate diffusion of the test substance into the receptor fluid such that the rate of diffusion into the receptor fluid did not become a rate-limiting step. The integrity of each membrane was assessed by measurement of electrical resistance prior to application of test substance. Following overnight skin membrane equilibration, test concentrations were applied *via* the donor chamber as a single application distributed evenly over the exposure area (0.64 cm^2).

The test substance, Indoxacarb 150 g/L EC, was applied as a 0.4 and 0.04 g a.s./L aqueous dilution and as the undiluted concentrate at 150 g a.s./L. Penetration and absorption were followed using [^{14}C]indoxacarb, which was uniformly blended into the formulations prior to application. The formulated products were applied at a rate of 10 μL/cm^2 to one group of eight skins per dose level per species. The amount of indoxacarb applied per area of skin was approximately 1500 μg/cm^2, 4 μg/cm^2, and 0.4 μg/cm^2 for the 150 g/L undiluted concentrate, 0.4 g/L aqueous spray dilution, and the 0.04 g/L aqueous spray dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. At 6 hours, the skin surface of all groups was washed and maintained until 18 hours post-exposure and then terminated. At termination, the application skin site was washed again and tape-stripped to remove the *stratum corneum* and total distribution of the applied material determined. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

One replicate each for rat and human skin was excluded in the undiluted concentrate dose group. The rat skin replicate was excluded as an outlier for absorbed dose ($p < 0.001$), likely caused by a failure of the skin integrity that occurred after the skin integrity measurement. The human skin replicate was excluded as an outlier due to low total percent recovery ($p < 0.0001$), likely caused by low recovery in the skin wash sample. Therefore, the sample size for both species was 7 represented by four individuals for both rat and human skin.

II. RESULTS AND DISCUSSION

A. INDOXACARB 150 g/L EC, 0.4 g a.s./L AQUEOUS DILUTION

The mean absorption of [^{14}C]indoxacarb into the receptor fluid over the 24-hour study duration was 1.67 μg equiv/cm^2 for rat skin and 0.187 μg equiv/cm^2 for human skin, representing 43.1% and 5.03% of the applied

dose in rat and human skin, respectively. The mean maximum penetration rate for the absorption of [^{14}C]indoxacarb through rat and human skin was 0.17 and 0.017 µg equiv/cm^2/h, respectively. The mean lag times were 0.63 hours in rat skin and 0.42 hours in human skin.

Greater than 75% of the absorption of indoxacarb in the receptor fluid over 24 hours occurred within half the study duration for rat skin. Therefore, all tape strips would be considered as unabsorbed dose. Nevertheless, less than 75% of the absorption of indoxacarb in the receptor fluid over 24 hours occurred within half the study duration for human skin. Thus the amount in the tape strips (except for the first two tape strips) was included as absorbed dose for human skin but also for rat skin in order to stay under the same conditions.

The mean recovery of [^{14}C]indoxacarb in rat and human skin was 96.5 ± 1.5% and 102 ± 12%, respectively.

Table B.6.2-8
Penetration kinetics of [^{14}C]indoxacarb from Indoxacarb 150 g/L EC, 0.4 g a.s./L aqueous dilution, 1-24 hours

Time (hour)	Data expressed in cumulative µg equiv./cm^2			
	Rat		Human	
	Mean	SD	Mean	SD
1	0.102	0.126	0.00820	0.00375
2	0.230	0.094	0.0261	0.0141
4	0.574	0.194	0.0430	0.0196
6 (end exposure)	0.859	0.242	0.0596	0.0270
12	1.30	0.27	0.105	0.047
24 (18 hours post-exposure)	1.67	0.23	0.187	0.093
Maximal flux (µg equiv/cm^2/h)	0.17	0.06	0.017	0.012
Lag time (h)	0.63	0.16	0.42	0.21

Table B.6.2-9
Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 0.4 g a.s./L aqueous dilution of Indoxacarb 150 g/L EC

	Data expressed as a percent of applied dose			
	Rat		Human	
	Mean	SD	Mean	SD
Receptor fluid	43.1	5.1	5.03	2.51
Donor chamber rinse	0.148	0.242	2.09	0.83
Tape strip total	3.04	3.19	42.7	14.5
Skin wash (6 h)	36.1	3.0	35.8	9.6
Skin wash (24 h)	4.01	1.53	9.32	1.98
(Stripped) skin	10.2	3.5	6.68	5.24
Total recovery	96.5	1.5	102	12
Absorbed dose without tape strips[a]	53.2	5.2	11.7	5.5
Absorbed dose with tape strips 3-10[b]	**54.7**	4.1	31.1	12.2
Total absorbed dose corrected for high variability	Correction not necessary		**43.3**	
Total absorbed dose corrected for low recovery	Correction not necessary		Correction not necessary	

[a] The percent of applied dose detected in the receptor fluid and skin, excluding all tape strips.

[b] The percent of applied dose detected in the receptor fluid and skin, excluding tape strips 1 & 2.

Table B.6.2-10
Percent distribution in tape strips (*stratum corneum*) at 24 hours following a 6-hour topical exposure to a 0.4 g a.s./L aqueous dilution of Indoxacarb 150 g/L EC

	Rat		Human	
	Mean	SD	Mean	SD
Tape strip 1	0.944	0.943	13.9	7.4
Tape strip 2	0.538	0.596	9.36	4.16
Tape strip 3	0.427	0.569	5.57	2.73
Tape strip 4	0.215	0.255	3.88	1.46
Tape strip 5	0.236	0.285	3.07	1.31
Tape strip 6	0.143	0.132	2.18	1.13
Tape strip 7	0.164	0.245	1.57	0.71
Tape strip 8	0.133	0.200	1.21	0.57
Tape strip 9	0.108	0.103	0.883	0.487
Tape strip 10	0.132	0.189	1.00	0.86
Total tape strips 3 to 10	1.558		19.363	

B. INDOXACARB 150 g/L EC, 0.04 g a.s./L AQUEOUS DILUTION

The mean absorption of [^{14}C]indoxacarb into the receptor fluid over the 24-hour study duration was 0.145 µg equiv/cm^2 for rat skin and 0.00979 µg equiv/cm^2 for human skin, representing 38.4% and 2.66% of the applied dose in rat and human skin, respectively. The mean maximum penetration rate for the absorption of [^{14}C]indoxacarb through rat and human skin was 0.016 and 0.0013 µg equiv/cm^2/h, respectively. The mean lag times were 0.69 hours in rat skin and 5.0 hours in human skin.

Greater than 75% of the absorption of indoxacarb in the receptor fluid over 24 hours occurred within half the study duration for rat skin. Therefore, all tape strips would be considered as unabsorbed dose. Nevertheless, less than 75% of the absorption of indoxacarb in the receptor fluid over 24 hours occurred

within half the study duration for human skin. Thus the amount in the tape strips (except for the first two tape strips) was included as absorbed dose for human skin but also for rat skin in order to stay under the same conditions.

The mean recovery of [^{14}C]indoxacarb in rat and human skin was 103 ± 10% and 100 ± 3%, respectively.

Table B.6.2-11
Penetration kinetics of [^{14}C]indoxacarb from Indoxacarb 150 g/L EC, 0.04 g a.s./L aqueous dilution, 1–24 hours

Time (hour)	Data expressed in cumulative µg equiv./cm^2			
	Rat		Human	
	Mean	SD	Mean	SD
1	0.00431	0.00500	NA	NA
2	0.0209	0.0109	NA	NA
4	0.0519	0.0237	0.00137	NA
6 (end exposure)	0.0744	0.0304	0.00189	NA
12	0.115	0.038	0.00629	0.00346
24 (18 hours post-exposure)	0.145	0.041	0.00979	0.00539
Maximal flux (µg equiv/cm^2/h)	0.016	0.007	0.0013	0.0009
Lag time (h)	0.69	0.30	5.0	1.9

Table B.6.2-12
Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 0.04 g a.s./L aqueous dilution of Indoxacarb 150 g/L EC

	Data expressed as a percent of applied dose			
	Rat		Human	
	Mean	SD	Mean	SD
Receptor fluid	38.4	10.1	2.66	1.46
Donor chamber rinse	0.294	0.340	3.14	2.05
Tape strip total	1.09	0.69	37.7	11.3
Skin wash (6 h)	51.1	10.8	37.2	13.2
Skin wash (24 h)	4.14	1.42	11.2	3.6
(Stripped) skin	8.11	2.96	7.58	5.37
Total recovery	103	10	100	3
Absorbed dose without tape strips[a]	46.5	10.0	10.2	5.8
Absorbed dose with tape strips 3-10[b]	**46.8**	10.0	25.7	10.9
Total absorbed dose corrected for high variability	Correction not necessary		**36.6**	
Total absorbed dose corrected for low recovery	Correction not necessary		Correction not necessary	

[a] The percent of applied dose detected in the receptor fluid and skin, excluding all tape strips.

[b] The percent of applied dose detected in the receptor fluid and skin, excluding tape strips 1 & 2.

Table B.6.2-13
Percent distribution in tape strips (*stratum corneum*) at 24 hours following a 6-hour topical exposure to a 0.04 g a.s./L aqueous dilution of Indoxacarb 150 g/L EC

	Rat		Human	
	Mean	**SD**	**Mean**	**SD**
Tape strip 1	0.540	0.382	14.7	4.4
Tape strip 2	0.152	0.106	7.54	3.22
Tape strip 3	0.0922	0.0786	4.96	2.26
Tape strip 4	0.0696	0.0486	2.99	1.23
Tape strip 5	0.0504	0.0328	2.30	0.67
Tape strip 6	0.0392	0.0303	1.55	0.49
Tape strip 7	0.0425	0.0355	1.55	0.54
Tape strip 8	0.0360	0.0205	1.35	0.61
Tape strip 9	0.0362	0.0216	1.14	0.45
Tape strip 10	0.0323	0.0195	0.915	0.443
Total tape strips 3 to 10	0.3984		16.755	

C. INDOXACARB 150 g/L EC, 150 g a.s./L UNDILUTED CONCENTRATE

The mean absorption of [^{14}C]indoxacarb into the receptor fluid over the 24-hour study duration was 139 µg equiv/cm^2 for rat skin and 7.81 µg equiv/cm^2 for human skin, representing 8.83% and 0.521% of the applied dose in rat and human skin, respectively. The mean maximum penetration rate for the absorption of [^{14}C]indoxacarb through rat and human skin was 16 and 0.52 µg equiv/cm^2/h, respectively. The mean lag times were 0.11 hours in rat skin and 1.2 hours in human skin.

Greater than 75% of the absorption of indoxacarb in the receptor fluid over 24 hours occurred within half the study duration for rat skin. Therefore, all tape strips would be considered as unabsorbed dose. Nevertheless, less than 75% of the absorption of indoxacarb in the receptor fluid over 24 hours occurred within half the study duration for human skin. Thus the amount in the tape strips (except for the first two tape strips) was included as absorbed dose for human skin but also for rat skin in order to stay under the same conditions.

The mean recovery of [^{14}C]indoxacarb in rat and human skin was $98.7 \pm 1.4\%$ and $92.8 \pm 1.7\%$, respectively.

Table B.6.2-14
Penetration kinetics of [^{14}C]indoxacarb from Indoxacarb 150 g/L EC, 150 g a.s./L undiluted concentrate, 1–24 hours

Time (hour)	Data expressed in cumulative µg equiv./cm^2			
	Rat		Human	
	Mean	SD	Mean	SD
1	14.8	3.2	NA	NA
2	31.0	7.4	0.399	0.530
4	60.1	15.0	1.48	1.21
6 (end exposure)	85.3	21.0	2.59	2.04
12	117	32	4.73	4.18
24 (18 hours post-exposure)	139	41	7.81	7.99
Maximal flux (µg equiv/cm^2/h)	16	4	0.52	0.39
Lag time (h)	0.11	0.06	1.2	0.6

Table B.6.2-15
Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 150 g a.s./L undiluted concentrate of Indoxacarb 150 g/L EC

	Data expressed as a percent of applied dose[a]			
	Rat		Human	
	Mean	SD	Mean	SD
Receptor fluid	8.83	2.53	0.521	0.526
Donor chamber rinse	0.651	0.831	0.0704	0.0447
Tape strip total	2.01	1.15	6.99	4.38
Skin wash (6 h)	82.1	7.8	81.5	7.8
Skin wash (24 h)	1.52	0.63	1.77	1.68
(Stripped) skin	3.52	3.36	1.99	1.40
Total recovery	98.7	1.4	92.8	1.7
Absorbed dose[a]	12.3	5.6	2.51	1.88
Absorbed dose with tape strips 3-10[b]	13.3	6.05	5.61	3.68
Total absorbed dose corrected for high variability	**19.35**		9.29	
Total absorbed dose corrected for low recovery	Correction not necessary		**10.01**	

[a] The percent of applied dose detected in the receptor fluid and skin, excluding all tape strips.

[b] The percent of applied dose detected in the receptor fluid and skin, excluding tape strips 1 & 2.

Table B.6.2-16
Percent distribution in tape strips (*stratum corneum*) at 24 hours following a 6-hour topical exposure to a 150 g a.s./L undiluted concentrate of Indoxacarb 150 g/L EC

	Rat		Human	
	Mean	**SD**	**Mean**	**SD**
Tape strip 1	0.679	0.254	2.62	1.66
Tape strip 2	0.415	0.269	1.26	1.05
Tape strip 3	0.243	0.161	0.851	0.619
Tape strip 4	0.159	0.109	0.558	0.453
Tape strip 5	0.124	0.085	0.468	0.342
Tape strip 6	0.107	0.098	0.363	0.232
Tape strip 7	0.0939	0.0865	0.255	0.132
Tape strip 8	0.0693	0.0595	0.261	0.142
Tape strip 9	0.0684	0.0612	0.192	0.122
Tape strip 10	0.0490	0.0413	0.153	0.079
Total tape strips 3 to 10	0.9136		3.101	

III. CONCLUSIONS

The results obtained in this study, using an *in vitro* dermal static diffusion cell model, demonstrate that penetration rate and percent absorption of indoxacarb from the Indoxacarb 150 g/L EC formulation, when applied either as an aqueous dilution or as the undiluted concentrated emulsifiable concentrate, was greatest for rat skin compared to human skin. After correcting for instances of low recovery (<95%) or high variability (standard deviation >25% of mean), the mean absorbed dose after 18 hours post-exposure was 19.35% in rat skin and 10.01% in human skin for the undiluted concentrate, 54.7% in rat skin and 43.3% in human skin for the 0.4 g indoxacarb/L aqueous spray dilution, and 46.8% in rat skin and 36.6% in human skin for the 0.04 g a.s./L aqueous spray dilution. The mean maximum penetration rates following sample application were 16 and 0.52 µg equiv/cm^2/h for undiluted Indoxacarb 150 g/L EC, 0.17 and 0.017 µg equiv/cm^2/h for the 0.4 g a.s./L aqueous spray dilution, and 0.016 and 0.0013 µg equiv/cm^2/h for the 0.04 g a.s./L aqueous spray dilution in rat and human skin, respectively.

B.6.3. AVAILABLE TOXICOLOGICAL DATA RELATING TO CO-FORMULANTS

Safety data sheets (SDS) for each co-formulant were provided by the applicant. The classification for human health of each co-formulant is available in Volume 4. Additional data to that contained in the safety data sheets is not currently available to the applicant.

B.6.4. EXPOSURE DATA

The following table presents the critical GAP used in the exposure assessments in this section.

Table B.6.4-1
Summary of critical use patterns (worst-case)

Crop	Application rate (kg a.s./ha)	Spray dilution (L/ha)	Application equipment	Number applications	Interval between applications
Maize, sweet corn	0.0375	100-1000	Groundboom/hydraulic nozzles	2	20 days
Lettuce		200-1000		4	7 days

B.6.4.1. Operator exposure

Operator exposure was estimated using the following exposure models:

German model: *Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection): Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft, Berlin-Dahlem, no. 277, 1992*

UK model: *Predictive Operator Exposure Model (POEM), UK MAFF, 1992, (revised 2007)*

Exposures were estimated using dermal absorption values of 2 and 18% for the concentrated product and diluted spray, respectively, and compared to the systemic AOEL of 0.003 mg/kg bw/day proposed in the context of indoxacarb renewal. Calculation results are presented for a 10-liter wide-mouth bottle. The results are provided in the following table. For other pack sizes (1, 2, 10, and 20 liter), calculations showed that exposure is almost comparable with 10-liter pack size.

Table B.6.4.1-1
Estimated operator exposure to indoxacarb

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of AOEL
Tractor boom sprayer application outdoors to field-grown crops			
German Model 20 ha/day 70 kg operator	no PPE	0.0045	149 %
UK POEM 50 ha/day, 6 h/day 100 L/ha 60 kg operator		0.0521	1737 %
German Model 20 ha/day 70 kg operator	Gloves during mixing/loading and application, coverall and sturdy footwear	0.0003	10 %
UK POEM 50 ha/day, 6 h/day 100 L/ha 60 kg operator	Gloves during mixing/loading and application	0.0081	271 %

Calculations

The actual calculations of the estimations of operator exposure are provided in Appendix 1.

Conclusion

According to the German model, it can be concluded that the risk for the operator using Indoxacarb 150 g/L EC on field-grown crops is acceptable with the use of personal protective equipment.

For information purposes only, the operator exposure has been estimated according to the EFSA calculator (Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874). Although the risk assessment using the EFSA model was not yet adopted at the time of the submission of the renewal dossier, the assessment of the renewal of preparations would have to be performed with this model.
The acute systemic exposure was assessed against the proposed AAOEL (see Volume 1 Level 2.6).
Parameters used in the model and detailed calculations are available in Appendix 3.

Table B.6.4.1-2
Estimated longer term systemic operator exposure

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of AOEL
Maize and lettuce			
EFSA calculator 50 ha/day 60 kg operator	potential	0.0059	198 %
	work wear M/L and A	0.0036	121 %
	work wear M/L and A + gloves M/L	0.0010	34 %
	work wear M/L and A + gloves M/L and A	0.0004	13 %

Table B.6.4.1-3
Estimated acute systemic operator exposure

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of AAOEL
Maize and lettuce			
EFSA calculator 50 ha/day 60 kg operator	potential	0.0526	1753 %
	work wear M/L and A	0.0215	717 %
	work wear M/L and A + gloves M/L	0.0119	398 %
	work wear M/L and A + gloves M/L and A	0.0118	394 %

B.6.4.2. Bystander and resident exposure

Bystander and resident exposures were estimated using the following exposure model:

- German model: *S. Martin, D. Westphal, M. Erdtmann-Vourliotis, F. Dechet, C. Schulze-Rosario, F. Stauber, H. Wicke and G. Chester, "Guidance for Exposure and Risk Evaluation for Bystanders and Residents Exposed to Plant Protection Products During and After Application," Journal of Consumer Protection and Food Safety 3 (2008) 272-281.*

The bystander exposure was also estimated using EUROPOEM II. An estimation was also provided by the applicant using the UK model (*Bystander Exposure Guidance. UK CRD Regulatory Update 10/2008, April 22, 2008).*

1- EUROPOEM II

A bystander may be exposed to spray drift during the tractor-mounted applications. Bystander exposure is estimated using EUROPOEM II data assuming the parameters outlined in the following Table B.6.4.2-1; the results of the exposure calculations are presented in Table B.6.4.2-2.

Table B.6.4.2-1: Parameters applied for the assessment of bystander exposure

Parameters and units	

	Application rate (g a.i./ha):	37.5
	Min. application volume (L/ha)	100
AR	Maximum Application rate (mg/m^2)	3.75
C	Maximum Spray concentration (mg a.i./mL)	0.375
DA	Dermal absorption of diluted formulation (%)	18
D	Drift at a distance of 7 m (% of applied dose) – worst-case for maize crops	2.14%
BSE	Exposed body surface (m^2)	1
BW	Body weight (kg)	60
IE	Inhalation exposure (mL/hr) (90^{th} percentile)#	0.03
T	Duration of exposure (hr)	0.083

based on 0.03 mL spray/m^3 (90^{th} percentile for field crops) and a breathing rate of 1 m^3/hr

Table B.6.4.2-2: Estimated bystander exposure

Parameters	
Dermal exposure (mg/person/day) = AR x D x BSE	0.08025
Dermal absorbed dose (mg/person/day) = dermal exposure x DA	0.01445
Inhalation exposure (mg/person/day) = IE x T x C	0.00093
Total systemic exposure (mg/person/day) = dermal absorbed dose + inhalation exposure	0.01538
Total systemic exposure (mg/kg bw/day) = total systemic exposure/BW	0.00026
% of AOEL	8.5%

Exposure of a bystander amounts to 8.5% of the AOEL of indoxacarb. The risk to bystander exposed to Indoxacarb 150 g/L EC is thus acceptable.

2- German model (Martin *et al.)*

Bystander and resident exposure have been calculated according to Martin *et al*. (2008). The maximum application rate for Indoxacarb 150 g/L EC of 0.0375 kg indoxacarb/ha was used for the calculations.

- **Bystander exposure**

Bystander exposure for adults and children after application of Indoxacarb 150 g/L EC was calculated according to Martin *et al*. (2008) and the results are summarised in the following table. The detailed calculations can be found in Appendix 2.

Table B.6.4.2-3
Bystander exposure after application of Indoxacarb 150g/L EC

	Adults (mg/kg bw/day)	Children (mg/kg bw/day)
Dermal exposure	0.0000326	0.0000255
Inhalation exposure	0.0000002	0.0000004
Total systemic exposure	0.0000328	0.0000258
% of AOEL	1.1	0.9

- **Resident exposure**

In addition to bystander, resident exposure for adults and children of Indoxacarb 150 g/L EC was calculated according to Martin *et al*. (2008), and the results are summarised in the following table. The detailed calculations can be found in Appendix 2.

Table B.6.4.2-4
Resident exposure after application of Indoxacarb 150g/L EC

	Adults (mg/kg bw/day)	Children (mg/kg bw/day)
Dermal exposure, absorbed dose	0.0000024	0.0000032
Inhalation exposure, absorbed dose	0.0002762	0.0005146
Oral exposure (hand-to-mouth), absorbed dose		0.0000008
Oral exposure (object-to-mouth), absorbed dose		0.0000002
Total systemic exposure	0.0002785	0.0005187
% of AOEL	9.3	17.3

3- UK model

This section represents applicant assessment, but the AOEL and dermal absorption values proposed by the RMS in the context of the renewal of indoxacarb were used.

For information, an assessment was proposed by the applicant using the UK model. Bystander and resident exposure have been estimated according to UK CRD guidance (2008). Exposure estimates from drift, vapour, and drift fallout are presented based on application using a field crop sprayer at an application rate of 37.5 g a.s./ha and a spray volume of 100 L/ha. Surface residue level from drift fallout is based on the conservative assumption that the residue accumulates with multiple (4 × 37.5 g a.s/ha) applications and does not dissipate. The calculation for exposure from vapour uses a very conservative potential vapour concentration of 1 $\mu g/m^3$. The vapour pressure of indoxacarb is approximately 3 orders of magnitude lower than the surrogate chemicals in the CRD guidance.

A summary of the exposure calculation results is presented in the following table using the AOEL and dermal absorption values proposed by the RMS in the context of the renewal. Actual calculations as provided by the applicant (taking into account AOEL and dermal absorption values different than those proposed by the RMS) are found in Appendix 2.

Table B.6.4.2-5
Estimation of bystander exposure from application of Indoxacarb 150 g/L EC

Exposure scenario		Total absorbed dose (mg/kg bw/day)	Percent of AOEL
Drift:		0.00015	5
Vapor:	Adult	0.00025	8
	Child	0.00055	18
Child:	Dermal	0.000094	
	Hand-to-mouth	0.000020	
	Object-to-mouth	0.000005	
	Total child	0.000119	4

Conclusion

Since the maximum application rate of Indoxacarb 150 g/L EC was used in the calculations, it can be concluded that there is no unacceptable risk anticipated for bystanders or residents due to exposure to indoxacarb from the agricultural use of Indoxacarb 150 g/L EC, according to the proposed GAP and label recommendations.

4- For information purposes only, the bystander and resident exposures have been estimated according to the EFSA calculator (Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874). Although the risk assessment using the EFSA model was not yet adopted at the time of the submission of the renewal dossier, the assessment of the renewal of preparations would have to be performed with this model.
The bystander systemic exposure was assessed against the proposed AAOEL (see Volume 1 Level 2.6).

Parameters used in the model and calculations are available in Appendix 3.

Table B.6.4.2-6
Estimated bystander exposure

	Adult		Child	
Model data	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**
Maize				
Spray drift (95th percentile)	0.0011	37 %	0.0041	138 %
Vapour (95th percentile)	0.0002	7.7 %	0.0011	36 %
Surface deposits (95th percentile)	0.0002	7.5 %	0.0006	19 %
Entry into treated crops (95th percentile)	0.0010	34 %	0.0019	62 %
Lettuce				
Spray drift (95th percentile)	0.0006	19 %	0.0021	69 %
Vapour (95th percentile)	0.0002	7.7 %	0.0011	36 %
Surface deposits (95th percentile)	0.0004	15 %	0.0011	37 %

Entry into treated crops (95th percentile)	0.0020	67 %	0.0036	121 %

Table B.6.4.2-7
Estimated resident exposure

	Adult		Child	
Model data	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Maize				
Spray drift (75th percentile)	0.0004	15 %	0.0018	61 %
Vapour (75th percentile)	0.0002	7.7 %	0.0011	36 %
Surface deposits (75th percentile)	0.0001	2.5 %	0.0002	6.3 %
Entry into treated crops (75th percentile)	0.0010	34 %	0.0019	62 %
All pathways (mean)	0.0013	44 %	0.0037	123 %
Lettuce				
Spray drift (75th percentile)	0.0002	7.2 %	0.0009	30 %
Vapour (75th percentile)	0.0002	7.7 %	0.0011	36 %
Surface deposits (75th percentile)	0.0001	4.9 %	0.0004	12 %
Entry into treated crops (75th percentile)	0.0020	67 %	0.0036	121 %
All pathways (mean)	0.0021	68 %	0.0047	158 %

B.6.4.3. Worker exposure

Worker exposure was calculated using the following exposure model:

EUROPOEM: *EUROPOEM II, 2002: Report of the Re-Entry Working Group – Post-Application Exposure of Workers to Pesticides in Agriculture. EUROPOEM II Project FAIR3-CT96-1406, December, 2002.*

Estimations are presented for post-application exposure of Indoxacarb 150 g/L EC in lettuce and maize. Since field worker exposure is possible during certain tasks, a conservative estimate using the approach presented by the EUROPOEM II report can be used to estimate potential worker exposure.

Table B.6.4.3-1
Parameters applied for the assessment of worker exposure

Parameters and units		**Maize**	**Lettuce**
DFR	Dislodgeable Foliar Residues (µg/cm²/kg a.i./ha) (default value)	3	3

AR	Application rate (kg a.i./ha)	0.0375 for 1 application 0.06 for 2 applications*	0.0375 for 1 application 0.12 for 4 applications**
TC	Transfer coefficient (cm²/ person/h)	10000	5000
T	Task duration (h)	2	8
P	Penetration through clothing factor	1 without PPE 0.1 with PPE	1 without PPE 0.1 with PPE
BW	Body weight (kg)	60	60
DA	Dermal absorption (%)	18%	18%

* MAF (multiple application factor) of 1.6 according to EFSA Guidance document 2014
** MAF of 3.2 according to EFSA Guidance document 2014

The potential dermal exposure of a worker is calculated by the following approach:

D (mg/person/d) = 0.001 x DFR (µg/cm²/kg a.i./ha) x AR (kg a.i./ha) x TC (cm²/person/h) x T (h/day) x P

As the potential inhalation exposure is considered to be negligible (outdoor applications), the worker total systemic exposure is calculated as follows:

$$\textbf{Total systemic exposure}\ (\text{mg/kg bw/d}) = \frac{\text{D (mg/person/d) x DA}}{\text{BW (kg)}}$$

Table B.6.4.3-2
Estimated worker exposure

Parameters	Maize 1 application	Maize 2 applications Intended GAP	Lettuce 1 application*	Lettuce 4 applications* Intended GAP
Without PPE				
Dermal exposure (mg/person/day) = D = 0.001 x DFR x AR x TC x T x P	2.25	3.6	4.5	14.4
Total systemic exposure (mg/kg bw/day) = (D x DA) / BW	0.00675	0.0108	0.0135	0.0432
% of AOEL	**225%**	**360%**	**450%**	**1440%**
With PPE				
Dermal exposure (mg/person/day) = D = 0.001 x DFR x AR x TC x T x P	0.225	0.36	0.45	1.44
Total systemic exposure (mg/kg bw/day) = (D x DA) / BW	0.000675	0.00108	0.00135	0.0043
% of AOEL	**23%**	**36%**	**45%**	**144%**

* Estimated worker exposure:
- considering 2 applications (MAF = 1.9 according to EFSA Guidance document 2014): 855% of the AOEL without PPE/**86%** of the AOEL with PPE
- considering 3 applications (MAF = 2.6 according to EFSA Guidance document 2014): 1170% of the AOEL without PPE/117% of the AOEL with PPE

Worker exposure is estimated to be >100% of the AOEL of indoxacarb for the unprotected worker in maize and lettuce crops. Worker exposure also exceeded 100% of the AOEL for the worker wearing PPE re-entering lettuce crops treated 4 times, as proposed by the applicant.
Exposure is estimated to be <100% of the AOEL of indoxacarb in maize crops for the worker wearing PPE, and in lettuce crops for the worker wearing PPE if the maximum number of applications is 2.

A dislodgeable foliar residue (DFR) study (Guinivan, 1997), performed with a different formulation (DPX JW062 60WG) was provided by the applicant. Nevertheless, as this study was conducted on apple leaves, the extrapolation to representative uses (lettuce and maize crops) is not considered acceptable. Therefore, the summary submitted by the applicant is provided in Annex 4 for information only.

For information purposes only, the worker exposure has been estimated according to the EFSA calculator (Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874). Although the risk assessment using the EFSA model was not yet adopted at the time of the submission of the renewal dossier, the assessment of the renewal of preparations would have to be performed with this model.

Parameters used in the model and detailed calculations are available in Appendix 3.

Table B.6.4.3-3
Estimated worker exposure

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of systemic AOEL
Maize: Inspection, irrigation Number of applications = 2, application interval = 20 days			
2 hours/day, Body weight: 60 kg	potential TC: 12500 cm^2/person/h	0.0138	458 %
	work wear TC: 1400 cm^2/person/h	0.0015	51 %
	work wear and gloves TC: no data	-	-
Lettuce: Reaching, picking Number of applications = 4, application interval = 7 days			
8 hours/day, Body weight: 60 kg	potential TC: 5800 cm^2/person/h	0.0500	1665 %
	work wear TC: 2500 cm^2/person/h	0.0215	718 %
	work wear and gloves TC: 580 cm^2/person/h	0.0050	167 %

B.6.5. EXPOSURE AND RISK ASSESSMENT

Operator:
According to the German model, for tractor mounted boom sprayer application in field, there is no unacceptable risk anticipated for operator wearing PPE.
For information, according to EFSA calculator, there is no unacceptable longer term risk anticipated for operator wearing PPE whereas the acute risk assessment showed exposure greater than the proposed AAOEL even with the use of PPE.

Bystander:
For all the intended uses, there is no unacceptable risk anticipated for a bystander incidentally exposed to Indoxacarb 150 g/L EC.
For information, according to EFSA calculator, there is no unacceptable risk anticipated for an adult bystander incidentally exposed to Indoxacarb 150 g/L EC, whereas an unacceptable risk is anticipated for a child.

Resident:
For all the intended uses, there is no unacceptable risk anticipated for a resident incidentally exposed to Indoxacarb 150 g/L EC.
For information, according to EFSA calculator, there is no unacceptable risk anticipated for an adult resident incidentally exposed to Indoxacarb 150 g/L EC, whereas an unacceptable risk is anticipated for a child.

Worker:
For maize, there is no unacceptable risk anticipated for the worker wearing PPE.
For lettuce, there is no unacceptable risk anticipated for the worker wearing PPE when re-entering crops treated **once or twice** with Indoxacarb 150 g/L EC. Nevertheless, in case of 4 applications on lettuce as proposed by the applicant, an unacceptable risk is anticipated for the worker even with the use of PPE.
For information, according to EFSA calculator, there is no unacceptable risk anticipated for worker wearing work wear when re-entering maize crops, whereas an unacceptable risk is anticipated for worker wearing PPE when re-entering lettuce crops.

As a conclusion, according to current used models, there is no unacceptable risk anticipated for the operator wearing PPE, the bystander and the resident for all of the intended uses of Indoxacarb 150 g/L EC.
Furthermore, no unacceptable risk is anticipated for the worker wearing PPE for maize. For lettuce, no unacceptable risk is anticipated for the worker wearing PPE if the maximum number of application is 2. In case of 4 applications on lettuce, as proposed by the applicant in the GAP table, an unacceptable risk is anticipated for the worker even with the use of PPE.

B.6.6. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CP, 7.1.1/01	[redacted]	2004	Indoxacarb (DPX-KN128)150 g/L EC: Acute oral toxicity in rodents - up-and-down procedure [redacted] DuPont-13455	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the	DuPont	N.A.[a]

			GLP: Yes Published: No			period of data protection has not expired at the time of submission of this dossier.		
CP, 7.1.2/01	[redacted]	2003	Indoxacarb (DPX-KN128) 150 g/L EC: Acute dermal toxicity study in rats [redacted] DuPont-13456 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 7.1.3/01	[redacted]	2004	Indoxacarb (DPX-KN128) 150 g/L EC: Inhalation median lethal concentration (LC_{50}) study in rats [redacted] DuPont-13460 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 7.1.4/01	[redacted]	2003	Indoxacarb (DPX-KN128) 150 g/L EC: Acute dermal irritation study in rabbits [redacted] DuPont-13457 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of	DuPont	N.A.

						submission of this dossier.		
CP, 7.1.5/01	[redacted]	2003	Indoxacarb (DPX-KN128) 150 g/L EC: Acute eye irritation study in rabbits [redacted] DuPont-13459 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 7.1.6/01	[redacted]	2003	Indoxacarb (DPX-KN128) 150 g/L EC: Dermal sensitization - Magnusson-Kligman maximization method [redacted] DuPont-13458 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 7.1.7/01	[redacted]	2012a	Indoxacarb (DPX-KN128) 150 g/L EC (pure active substance indoxacarb) Repeated-dose oral toxicity 28-day feeding study in rats [redacted] DuPont-33601 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 7.1.7/02	[redacted]	2012b	Indoxacarb (DPX-KN128)	Y	Y	The study is necessary for	DuPont	N.A.

			150 g/L EC (pure active substance indoxacarb): Subchronic toxicity 90-day feeding study in rats [redacted] DuPont-33600 GLP: Yes Published: No			the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CP, 7.3/01	Mingoia, R.T.	2014a	Indoxacarb (DPX-KN128) 150 g/L EC: *In vitro* percutaneous absorption of indoxacarb in rat and human skin DuPont Haskell Laboratory DuPont-38096 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CP, 7.3/02	[redacted]	2014b	Indoxacarb (DPX-KN128) 150 g/L EC: *In vivo* dermal absorption of indoxacarb in the rat [redacted] DuPont-38097 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

[a] N.A. = not applicable, as this is a new study submitted for the first time at EU level for the purpose of renewal.

Appendix 1 – Estimations of operator exposure

German model – without PPE

THE GERMAN MODEL (GEOMETRIC MEAN VALUES)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**Indoxacarb 150g/L EC**		Active substance		**indoxacarb**
Formulation type	Liquid		a.s. concentration	**150**	g/l
Dermal absorption from product	**2**	%	Dermal absorption from spray	**18**	%
RPE during mix/loading	None		RPE during application	None	
PPE during mix/loading	None				
PPE during application: Head	None	Hands	None	Body	None
Dose	**0.25**	l product/ha	Work rate/day	**20**	ha

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.s.	2.4	mg/kg a.s.
Hand contamination/day	1.8	mg/day
Protective clothing	none	
Transmission to skin	100	%
Dermal exposure to a.s.	1.8	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.s.	0.0006	mg/kg a.s.
Inhalation exposure/day	0.00045	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.00045	mg/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique: Tractor-mounted/trailed boom sprayer: hydraulic nozzles

	Head	Hands	Rest of body	
Dermal contamination/kg a.s.	0.06	0.38	1.6	
Dermal contamination/day	0.045	0.285	1.2	
Protective clothing	none	none	none	
Transmission to skin	100	100	100	%
Total dermal exposure to a.s.	1.53	mg/day		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure/kg a.s.	0.001	mg/kg a.s.
Inhalation exposure/day	0.00075	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.00075	mg/day

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.s.	1.8	mg/day	1.53	mg/day
Percent absorbed	2	%	18	%
Absorbed dose (dermal route)	0.036	mg/day	0.2754	mg/day
Inhalation exposure to a.s.	0.00045	mg/day	0.00075	mg/day
Total systemic exposure	0.03645	mg/day	0.27615	mg/day

PREDICTED EXPOSURE

Total systemic exposure	0.3126	mg/day
Operator body weight	70	kg
Operator exposure	**0.0045**	**mg/kg bw/day**

AOEL (mg/kg/day) =	0.0030
% of AOEL =	**148.9%**

German model – with PPE

THE GERMAN MODEL (GEOMETRIC MEAN VALUES)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles					
Product	**Indoxacarb 150g/L EC**		Active substance		**indoxacarb**	
Formulation type	Liquid		a.s. concentration		**150**	g/l
Dermal absorption from product	**2**	%	Dermal absorption from spray		**18**	%
RPE during mix/loading	None		RPE during application		None	
PPE during mix/loading	Gloves					
PPE during application: Head	None	Hands	Gloves	Body	Coverall and sturdy footwear	
Dose	**0.25**	l product/ha	Work rate/day		**20**	ha

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.s.	2.4	mg/kg a.s.
Hand contamination/day	1.8	mg/day
Protective clothing	gloves	
Transmission to skin	1	%
Dermal exposure to a.s.	0.018	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.s.	0.0006	mg/kg a.s.
Inhalation exposure/day	0.00045	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.00045	mg/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique: Tractor-mounted/trailed boom sprayer: hydraulic nozzles

	Head	Hands	Rest of body	
Dermal contamination/kg a.s.	0.06	0.38	1.6	
Dermal contamination/day	0.045	0.285	1.2	
Protective clothing	none	gloves	coverall and sturdy footwear	
Transmission to skin	100	1	5	%
Total dermal exposure to a.s.	0.10785	mg/day		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure/kg a.s.	0.001	mg/kg a.s.
Inhalation exposure/day	0.00075	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.00075	mg/day

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.s.	0.018	mg/day	0.10785	mg/day
Percent absorbed	2	%	18	%
Absorbed dose (dermal route)	0.00036	mg/day	0.019413	mg/day
Inhalation exposure to a.s.	0.00045	mg/day	0.00075	mg/day
Total systemic exposure	0.00081	mg/day	0.020163	mg/day

PREDICTED EXPOSURE

Total systemic exposure	0.020973	mg/day
Operator body weight	70	kg
Operator exposure	**0.0003**	**mg/kg bw/day**

AOEL (mg/kg/day) =	0.0030
% of AOEL =	**10.0%**

UK-POEM – Without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	**Indoxacarb 150g/L EC**	Active substance	**indoxacarb**	
Formulation type	organic solvent-based	a.s. concentration	**150**	mg/ml
Dermal absorption from product	**2** %	Dermal absorption from spray	**18**	%
Container	10 litres 63 mm closure			
PPE during mix/loading	None	PPE during application	None	
Dose	**0.25** l/ha	Work rate/day	**50**	ha
Application volume	**100** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	0.25	litres product/ha
Work rate	50	ha/day
Number of operations	2	/day
Hand contamination	0.1	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.1	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.1	ml/day	41.55	ml/day
Concen. of a.s. product or spray	150	mg/ml	0.375	mg/ml
Dermal exposure to a.s.	15	mg/day	15.58125	mg/day
Percent absorbed	2	%	18	%
Absorbed dose	0.3	mg/day	2.804625	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.375	mg/ml
Inhalation exposure to a.s.	0.0225	mg/day
Percent absorbed	100	%
Absorbed dose	0.0225	mg/day

PREDICTED EXPOSURE

Total absorbed dose	3.127125	mg/day
Operator body weight	60	kg
Operator exposure	0.05211875	mg/kg bw/day

AOEL (mg/kg/day) =	0.0030
% of AOEL =	**1737.3%**

UK-POEM – With PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	**Indoxacarb 150g/L EC**	Active substance	**indoxacarb**	
Formulation type	organic solvent-based	a.s. concentration	**150**	mg/ml
Dermal absorption from product	**2** %	Dermal absorption from spray	**18**	%
Container	10 litres 63 mm closure			
PPE during mix/loading	Gloves	PPE during application	Gloves	
Dose	**0.25** l/ha	Work rate/day	**50**	ha
Application volume	**100** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	0.25	litres product/ha
Work rate	50	ha/day
Number of operations	2	/day
Hand contamination	0.1	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.01	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	5%	15%	
Dermal exposure	0.65	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.01	ml/day	6.45	ml/day
Concen. of a.s. product or spray	150	mg/ml	0.375	mg/ml
Dermal exposure to a.s.	1.5	mg/day	2.41875	mg/day
Percent absorbed	2	%	18	%
Absorbed dose	0.03	mg/day	0.435375	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.375	mg/ml
Inhalation exposure to a.s.	0.0225	mg/day
Percent absorbed	100	%
Absorbed dose	0.0225	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.487875	mg/day
Operator body weight	60	kg
Operator exposure	0.00813125	mg/kg bw/day

AOEL (mg/kg/day) =	0.0030
% of AOEL =	**271.0%**

Appendix 2 – Estimations of bystander/resident exposure

According to Martin et al. :

Estimation of bystander exposure during/after application in Field Crops, Tractor Mounted

Input parameters considered for the estimation of bystander exposure:

Intended use(s):			**Drift (D):**	0.29	% (FCTM, 10 m)
Application rate (AR):	0.0375	kg a.s./ha	**Exposed Body Surface Area (BSA):**	1	m² (adults)
				0.21	m² (children)
Body weight (BW):	60	kg/person (adults)	**Specific Inhalation Exposure (I^*_A):**	0.001	mg/kg a.s. (6 hours, adults)
	16.15	kg/person (children)		0.00057	mg/kg a.s. (6 hours, children)
Dermal absorption (DA):	18.00	% ('worst case')	**Area Treated (A):**	20	ha/d (based on Field Crops, Tractor Mounted (FCTM))
Inhalation absorption (IA):	100	%	**Exposure duration (T):**	5	min
AOEL:	0.003	mg/kg bw/d			

Bystander exposure towards indoxacarb					
Adults			**Children**		
Bystander: Dermal exposure after application in (via spray drift)					
SDE_B = (AR x D x BSA x DA) / BW			SDE_B = (AR x D x BSA x DA) / BW		
(3.75 x 0.29% x 1 x 18%) / 60			(3.75 x 0.29% x 0.21 x 18%) / 16.15		
External exposure	0.010875	mg/person	External exposure	0.00228375	mg/person
External exposure	0.00018125	mg/kg bw/d	External exposure	0.00014141	mg/kg bw/d
Absorbed dose:	**0.0000326**	**mg/kg bw/d**	**Absorbed dose:**	**0.0000255**	**mg/kg bw/d**
Bystander: Inhalation exposure after application in					
SIE_B = (I^*_A x AR x A x T x IA) / BW			SIE_B = (I^*_A x AR x A x T x IA) / BW		
(0,000 / 360 x 0.0375 x 20 x 5 x 100%) / 60			(0,000 / 360 x 0.0375 x 20 x 5 x 100%) / 16.15		
External exposure	1.0417E-05	mg/person	External exposure	5.9866E-06	mg/person
External exposure	1.7361E-07	mg/kg bw/d	External exposure	3.7069E-07	mg/kg bw/d
Absorbed dose:	**0.0000002**	**mg/kg bw/d**	**Absorbed dose:**	**0.0000004**	**mg/kg bw/d**
Total systemic exposure: SE_B = SDE_B + SIE_B			Total systemic exposure: SE_B = SDE_B + SIE_B		
Total systemic exposure (absorbed dose)	0.00196792	mg/person	Total systemic exposure (absorbed dose)	0.00041706	mg/person
Total systemic exposure (absorbed dose)	**0.0000328**	**mg/kg bw/d**	**Total systemic exposure** (absorbed dose)	**0.0000258**	**mg/kg bw/d**
% of AOEL:	**1.09**	**%**	**% of AOEL:**	**0.86**	**%**

Estimation of resident exposure after application in Field Crops, Tractor Mounted (FCTM)

Input parameters considered for the estimation of resident exposure:

Intended use(s):			**Drift (D):**	0.29	% (FCTM, 10 m)
Application rate (AR):	0.0375	kg a.s./ha	**Transfer coefficient (TC):**	7300	cm^2/h (adults)
				2600	cm^2/h (children)
Number of applications (NA):	1		**Turf Transferable Residues (TTR):**	5	%
Body weight (BW):	60	kg/person (adults)	**Exposure Duration (H):**	2	h
	16.15	kg/person (children)	**Airborne Concentration of Vapour (ACV):**	0.001	mg/m3
Dermal absorption (DA):	18.00	% ('worst case')	**Inhalation Rate (IR):**	16.57	m^3/d (adults),
Inhalation absorption (IA):	100	%		8.31	m^3/d (children)
Oral absorption (OA)	60	%	**Saliva Extraction Factor (SE):**	50	%
AOEL	0.003	mg/kg bw/d	**Surface Area of Hands (SA):**	20	cm^2
			Frequency of Hand to Mouth (Freq):	20	events/h
			Dislodgeable foliar residues (DFR):	20	%
			Ingestion Rate for Mouthing of Grass/Day (IgR):	25	cm^2/d

Resident exposure towards indoxacarb					
Adults			**Children**		
Residents: Dermal exposure after application in (via deposits caused by spray drift)					
SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW			SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW		
(0.000375 x 1 x 0.29% x 5% x 7300 x 2 x 18%) / 60			(0.000375 x 1 x 0.29% x 5% x 2600 x 2 x 18%) / 16.15		
External exposure	0.00079388	mg/person	External exposure	0.00028275	mg/person
External exposure	1.3231E-05	mg/kg bw/d	External exposure	1.7508E-05	mg/kg bw/d
Absorbed dose:	**0.0000024**	**mg/kg bw/d**	**Absorbed dose:**	**0.0000032**	**mg/kg bw/d**
Residents: Inhalation exposure to vapour					
SIE_R = (AC_V x IR x IA) / BW			SIE_R = (AC_V x IR x IA) / BW		
(0.001 x 16.57 x 100%) / 60			(0.001 x 8.31 x 100%) / 16.15		
External exposure	0.01657	mg/person	External exposure	0.00831	mg/person
External exposure	0.00027617	mg/kg bw/d	External exposure	0.00051455	mg/kg bw/d
Absorbed dose:	**0.0002762**	**mg/kg bw/d**	**Absorbed dose:**	**0.0005146**	**mg/kg bw/d**
			Residents: Oral exposure (hand-to-mouth transfer)		
			SOE_H = (AR x NA x D x TTR x SE x SA x Freq x H x OA) /		
			(0.000375 x 1 x 0.29% x 5% x 50% x 20 x 20 x 2 x 60%) / 16.15		
			External exposure	0.00002175	mg/person
			External exposure	1.3467E-06	mg/kg bw/d
			Absorbed dose	**0.0000008**	**mg/kg bw/d**
			Residents: Oral exposure (object-to-mouth transfer)		
			SOE_O = (AR x NA x D x DFR x IgR x OA) / BW		
			(0.000375 x 1 x 0.29% x 20% x 25 x 60%) / 16.15		
			External exposure	5.4375E-06	mg/person
			External exposure	3.3669E-07	mg/kg bw/d
			Absorbed dose	**0.0000002**	**mg/kg bw/d**
Total systemic exposure: SE_R = SDE_R + SIE_R			Total systemic exposure: SE_R = SDE_R + SIE_R + SOE_H + SOE_O		
Total systemic exposure (absorbed dose)	0.0167129	mg/person	Total systemic exposure (absorbed dose)	0.00837721	mg/person
Total systemic exposure (absorbed dose)	**0.0002785**	**mg/kg bw/d**	**Total systemic exposure** (absorbed dose)	**0.0005187**	**mg/kg bw/d**
% of AOEL:	**9.28**	**%**	**% of AOEL:**	**17.29**	**%**

According to UK model :
Assessment provided by the applicant using an AOEL of 0.004 mg/kg bw/d and a dermal absorption of 2%:

Exposure from Spray Drift							
Systemic Exposure = ((PDE x SC x DA) + (PIE x SC))/BW =						0.00005	mg/kg/day
Where:							
	PDE = potential dermal exposure (mL spray)					0.1	
	PIE = potential inhalation exposure (mL spray)					0.006	
	SC = concentration of active substance in spray (mg/mL)					0.375	
	DA = dermal absorption factor					0.02	
	BW = body weight (kg)					60	
Systemic Exposure =	0.000050	mg/kg/day					
Percent of AOEL =	(Systemic Exposure/AOEL) x 100 =					**1.3**	**%**

Exposure from Vapor							
Potential vapor concentration (µg/m³):				1			
Breathing rate and body weight assumed:							
	Adult = 15.2 m^3/day, 60 kg						
	Child = 8.3 m^3/day, 15 kg						
Potential Exposure = (Vapor conc. x Breathing rate)/BW							
	Adult =	0.253333	µg/kg/day	=	0.00025	mg/kg/day	
	Child =	0.553333	µg/kg/day	=	0.00055	mg/kg/day	
% of AOEL:							
	Adult =	(Potential Exposure/AOEL) x 100 =				**6.3**	**%**
	Child =	(Potential Exposure/AOEL) x 100 =				**13.8**	**%**

Exposure to Drift Fallout						
Child Dermal Exposure						
SE(d) = (AR x DF x TTR x TC x H x DA)/BW						
Where:						
	SE(d) = systemic exposure via the dermal route					
	AR = field application rate; seasonal maximum (µg/cm^2)					1.5
	DF = drift fallout (default estimate % of AR)					0.01
	TTR = turf transferable residue (default estimate)					0.05
	TC = transfer coefficient (cm^2/hr)					5200
	H = exposure duration (hr)					2
	DA = dermal absorption factor					0.02
	BW = body weight (kg)					15
SE(d) =	0.0104	µg/kg/day	=	0.000010	mg/kg/day	

Child Hand-to-Mouth Exposure							
SE(h) = (AR x DF x TTR x SE x SA x Freq x H)/BW							
Where:							
	SE(h) = systemic exposure via the dermal route						
	AR = field application rate; seasonal maximum (µg/cm^2)						1.5
	DF = drift fallout (default estimate % of AR)						0.01
	TTR = turf transferable residue (default estimate)						0.05
	SE = saliva extraction factor (default = 50%)						0.5
	SA = surface area of the hands (default = 20 cm^2)						20
	Freq = frequency of hand to mouth (default = 20 events/hr)						20
	H = exposure duration (hr)						2
	BW = body weight (kg)						15
SE(h) =	0.02	µg/kg/day	=	0.000020	mg/kg/day		

Child Object-to-Mouth Exposure							
SE(o) = (AR x DF x TTR x IgR)/BW							
Where:							
	SE(o) = systemic exposure via mouth activity						
	AR = field application rate; seasonal maximum (µg/cm ^{2})						1.5
	DF = drift fallout (default estimate % of AR)						0.01
	TTR = turf transferable residue (default estimate = 20%)						0.2
	IgR = ingestion rate for mouthing grass (default = 25 cm ^{2}/day)						25
	BW = body weight (kg)						15
SE(o) =	0.005	µg/kg/day	=	0.0000050	mg/kg/day		

Child Total Exposure							
SE(d) + SE(h) + SE(o) =			0.0354	µg/kg/day	=	0.000035	mg/kg/day
% of AOEL =		**0.9**					

Appendix 3 – Estimations of operator, resident, bystander and worker exposure according to EFSA calculator

- **Data entry for maize use:**

Substance name	Indoxacarb	
Product name	Indoxacarg 150g/L EC	
Reference value non acutely toxic active substance (RVNAS)	0.003	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)	0.003	mg/kg bw/day
Crop type	Cereals	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	100	L/ha
Maximum application rate of active substance	0.0375	kg a.s. /ha
50% Dissipation Time DT50	30	days
Initial Dislodgeable Foliar Residue	3	µg/cm2 of foliage/kg a.s. applied/ha
Dermal absorption of product	2.00%	
Dermal absorption of in-use dilution	18.00%	
Oral absorption of active substance	60.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Buffer strip	2-3	m
Number of applications	2	
Interval between multiple applications	20	days
Season (upward spraying orchards only)	not relevant	

- **Data entry for lettuce use:**

Substance name	Indoxacarb	
Product name	Indoxacarb 150g/L EC	
Reference value non acutely toxic active substance (RVNAS)	0.003	mg/kg bw/day
Reference value acutely toxic active substance (RVAAS)	0.003	mg/kg bw/day
Crop type	Leaf vegetables and fresh herbs	
Substance properties		
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Miniumum volume water for application (liquids)	200	L/ha
Maximum application rate of active substance	0.0375	kg a.s. /ha
50% Dissipation Time DT50	30	days
Initial Dislodgeable Foliar Residue	3	µg/cm2 of foliage/kg a.s. applied/ha
Dermal absorption of product	2.00%	
Dermal absorption of in-use dilution	18.00%	
Oral absorption of active substance	60.00%	
Inhalation absorption of active substance	100.00%	
Vapour pressure of active substance	low volatile substances having a vapour pressure of <5*10-3Pa	
Scenario		
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Buffer strip	2-3	m
Number of applications	4	
Interval between multiple applications	7	days
Season (upward spraying orchards only)	not relevant	

Exposure assessment for maize use:

Substance	Indoxacarb	Formulation = Soluble concentrates, emulsifiable concentrate, etc.	Application rate-0.0375 kg a.s. /ha	Spray dilution = 0.375 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 2, Application interval = 20 days
Percentage Absoprtion	Dermal for product = 2	Dermal for in use diluation = 18	Oral = 60	Inhalation = 100	
RVNAS	0.003 mg/kg bw/day		RVAAS	0.003 mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0059	% of RVNAS	197.66%
	Acute systemic exposure mg/kg bw/day		0.0526	% of RVAAS	1753.19%
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0004	% of RVNAS	12.51%
	Acute systemic exposure mg/kg bw/day		0.0118	% of RVAAS	393.53%

Worker - Inspection, irrigation	Potential exposure mg/kg bw/day	0.0138	% of RVNAS	458.43%
	Working clothing mg/kg bw/day	0.0015	% of RVNAS	51.34%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0018	% of RVNAS	60.61%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	35.67%
	Surface deposits (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	6.33%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0019	% of RVNAS	61.89%
	All pathways (mean) mg/kg bw/day	0.0037	% of RVNAS	123.07%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0004	% of RVNAS	14.47%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	7.67%
	Surface deposits (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	2.50%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0010	% of RVNAS	34.38%
	All pathways (mean) mg/kg bw/day	0.0013	% of RVNAS	43.79%

Bystander - child	Spray drift (95th percentile) mg/kg bw/day	0.0041	% of RVAAS	137.93%
	Vapour (95th percentile) mg/kg bw/day	0.0011	% of RVAAS	35.67%
	Surface deposits (95th percentile) mg/kg bw/day	0.0006	% of RVAAS	18.81%
	Entry into treated crops (95th percentile) mg/kg bw/day	0.0019	% of RVAAS	61.89%
Bystander - adult	Spray drift (95th percentile) mg/kg bw/day	0.0011	% of RVAAS	37.31%
	Vapour (95th percentile) mg/kg bw/day	0.0002	% of RVAAS	7.67%
	Surface deposits (95th percentile) mg/kg bw/day	0.0002	% of RVAAS	7.53%
	Entry into treated crops (95th percentile) mg/kg bw/day	0.0010	% of RVAAS	34.38%

Recreational Exposure	Child % of RVNAS	Adult % of RVNAS

Exposure assessment for lettuce use:

Substance	Indoxacarb	Formulation = Soluble concentrates, emulsifiable concentrate, etc.	Application rate-0.0375 kg a.s. /ha	Spray dilution = 0.1875 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Leaf vegetables and fresh herbs / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 4, Application interval = 7 days
Percentage Absoprtion	Dermal for product = 2	Dermal for in use diluation = 18	Oral = 60	Inhalation = 100	
RVNAS	0.003 mg/kg bw/day		RVAAS	0.003 mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0059	% of RVNAS	197.66%
	Acute systemic exposure mg/kg bw/day		0.0526	% of RVAAS	1753.19%
Mixing and Loading		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = Yes	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0004	% of RVNAS	12.51%
	Acute systemic exposure mg/kg bw/day		0.0118	% of RVAAS	393.53%

Worker - Reaching, picking	Potential exposure mg/kg bw/day	0.0500	% of RVNAS	1665.11%
	Working clothing mg/kg bw/day	0.0215	% of RVNAS	717.72%
	Working clothing and gloves mg/kg bw/day	0.0050	% of RVNAS	166.51%

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0009	% of RVNAS	30.30%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	35.67%
	Surface deposits (75th percentile) mg/kg bw/day	0.0004	% of RVNAS	12.39%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0036	% of RVNAS	121.12%
	All pathways (mean) mg/kg bw/day	0.0047	% of RVNAS	158.02%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	7.24%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	7.67%
	Surface deposits (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	4.89%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0020	% of RVNAS	67.29%
	All pathways (mean) mg/kg bw/day	0.0021	% of RVNAS	68.34%

Bystander - child	Spray drift (95th percentile) mg/kg bw/day	0.0021	% of RVAAS	68.97%
	Vapour (95th percentile) mg/kg bw/day	0.0011	% of RVAAS	35.67%
	Surface deposits (95th percentile) mg/kg bw/day	0.0011	% of RVAAS	36.81%
	Entry into treated crops (95th percentile) mg/kg bw/day	0.0036	% of RVAAS	121.12%
Bystander - adult	Spray drift (95th percentile) mg/kg bw/day	0.0006	% of RVAAS	18.66%
	Vapour (95th percentile) mg/kg bw/day	0.0002	% of RVAAS	7.67%
	Surface deposits (95th percentile) mg/kg bw/day	0.0004	% of RVAAS	14.74%
	Entry into treated crops (95th percentile) mg/kg bw/day	0.0020	% of RVAAS	67.29%

Recreational Exposure	Child % of RVNAS	Adult % of RVNAS

Appendix 4 – Estimations of worker exposure

Summary of the study as provided by the applicant

CA 7.2.3.2

Report: Guinivan, R.A., Enriquez, M.A., Schelling-Schiavo, M. (1997); Magnitude and decline pattern of KN128 together with KN127 foliar dislodgeable residues in/on apple leaves from crops grown in Europe following applications of DPX-JW062 WG - season 1995 (analysis by GC/MSD)

DuPont Report No.: AMR 3394-95

Guidelines: European Union Directive 911414/EEC, Residue Chemistry **Deviations:** None

Testing Facility: Battelle Europe-Centre de Recherche de Geneve, Geneva, Switzerland

Testing Facility Report/Project No.: A-11-96-18

GLP: Yes

Certifying Authority: Not specified

I. MATERIALS AND METHODS

The field program was conducted in 1995 at one location each in Germany and Italy. DPX-JW062 60 WG was applied six times foliarly at 50 g indoxacarb (DPX-KN128)/ha/application to apple trees (as representative of orchard crops), for a seasonal application rate of 300 g a.s./ha. The applications were made at 10-25 day intervals with the last application occurring approximately 28 days before normal harvest, according to the use pattern shown in the following table.

Table 1
Study Use Pattern

Trial Identification (Location, Country/Year)	EP[a]	Application						Tank mix adjuvants	Ref[e]
		Method/ timing	Vol,[b] (L/ha)	Rate (g ai/ha)[c]	Rate (g a.s./hL)	RTI[d] Days	Total rate (g a.s./ha)[c]		
Trial 01 (Machern, Saxony, Germany /1995)	60WG	Directed foliar/to maturing apples	1500	50.0	3.33	N/A	300	None	AMR 3394-98
			1500	50.0	3.33	13			
			1500	50.0	3.33	25			
			1500	50.0	3.33	10			
			1500	50.0	3.33	14			
			1500	50.0	3.33	11			
Trial 02 (Marega de Bevilacqua, Veneto, Italy/1995)	60WG	Directed foliar/to maturing apples	806.2	49.7	6.16	N/A	297.1	None	AMR 3394-98
			804.6	49.4	6.14	10			
			806.2	50.0	6.20	10			
			802.5	49.7	6.19	10			
			796.8	49.2	6.17	10			
			818.7	49.1	6.00	10			

[a] EP = End-use Product (DPX-JW062-133 WG, 30% KNI28).

[b] Litres/hectare.

[c] Grams active substance per hectare (expressed as KN128, the active insecticide).

[d] Retreatment Interval.

[e] Summarised this document

A total of two dislodgeable foliar residue trials were conducted in apples over one growing season (1995). A summary of these orchard trials is given below. Locations of the apple trial sites are given in Figure 1.

Figure 1
Map: Indoxacarb apple trial sites

Reference,	Trial No.	Location
AMR 3394-95	1	Machern, Saxony, Germany
AMR 3394-95,	2	Marega de Bevilacqua, Veneto, Italy

An apple dislodgeable residue data summary (in $\mu g/cm^2$) is presented in Table 2.

To generate these data, the following analysis and recovery information pertains.

Analysis method: Gas chromatography/mass spectrometry (GC/MSD) method (DuPont Report No. AMR 3493-95, Supplement No. 1) developed as the indoxacarb residue method for determining dislodged crop residues in dislodging solutions. The method was modified to include GC/MSD conditions and calibration and validated by Battelle under Study No. A-11-94-31.

Analyte:	Indoxacarb (total residue, DPX- KN128 and In-KN127 isomers analysed as a single chromatographic peak)
Extraction:	The leaf residues are dislodged by repeated shaking with aqueous 0.01% Aerosol®OT solution. Residues in the combined dislodging solutions are extracted with toluene, concentrated, and brought to volume in toluene.
Clean-up:	None required
Chromatography:	HP 5890 (series II) gas chromatograph / 5972 mass spectrometer detector, equipped with a HP 7673 automatic injector system
Detection:	GC Column: DB-SMS (crosslinked 5% PH methyl siloxane) HP column, 15 m × 0.25 mm ID, 0.25-um film thickness
Limit of Determination:	0.09 µg/mL (0.2 µg/100 mL of dislodging solution)

Storage stability: Samples from this study were stored at about -l8°C or below for periods up to a maximum of 12 months. The analytes (DPX-KN128/IN-KNI27) have been shown to be stable on many matrices stored at about -20°C for up to at least 15 months. Therefore, storage conditions were sufficient to maintain the integrity of the samples.

Table 2
Dislodgeable foliar residues of DPX-KN128 + IN-KN127 from apple supervised trials

Average dislodgeable foliar residues found at each sampling event		
	Indoxacarb residues (µg/cm2)[a]	
Sampling event	**Trial site 01 – Germany (uncorrected)[b]**	**Trial site 02 – Italy (uncorrected)[b]**
+ 3 hr post-app #6	0.21 [0.25, 0.15, 0.22]	0.22 [0.23, 0.20, 0.22]
+ 3 day post-app #6	0.08 [0.06, 0.07, 0.12]	0.12 [0.11, 0.12, 0.13]
+ 7 days post-app #6	0.06 [0.08, 0.07, 0.05]	0.05 [0.04, 0.04, 0.06]
+ 14 days post-app #6	0.04 [0.05, 0.04, 0.04]	0.03 [0.03, 0.03, 0.03]
+ 28 days post-app #6	0.03 [0.02, 0.04, 0.02]	0.02 [0.03, 0.02, 0.02]

[a] Average of three treated samples (the individual results are depicted in brackets)

[b] Corrected residue values were not presented. The kinetics evaluation was performed using mean uncorrected residues.

Recovery data: The method was validated prior to sample analysis. Recoveries of indoxacarb from three fortifications (0.20, 40 and 400 µg/100 mL dislodging solution) were 77, 111, and 98%, respectively (mean, 95.3%).

Recovery data for fortifications run concurrently with the treated samples are given in Table . The unfortified control samples, including untreated control field samples collected at 0, 7 and 28 DALA at each test location, showed no detectable residues. Data from the analysis of unfortified controls and fortified controls validated method performance.

Table 3
Summary of recoveries of indoxacarb from lab fortification dislodging solutions

Matrix	Fortification level		Sample size (n)	Recoveries (%)	Mean ± std. dev. (% ± %), RSD
	µg/100mL	Equiv. µg/cm2			
Concurrent fortifications – Indoxacarb					
Dislodging solution (leaf discs)	5	0.008	2	91, 92	91.5
	50	Not specified	2	82, 97	89.5
	100	Not specified	2	79, 104	91.5
	400	0.68	2	107, 77	92.0
Overall:	5-50	0.008-0.68	8	79-107	91.1 ± 11, 12

II. RESULTS AND DISCUSSION

The residue studies presented herein were carried out in the Germany and Italy and provide data relevant to conditions in orchard fruit-growing regions of the northern and southern Europe.

All of the analytical work associated with the studies was performed at Battelle, Geneva Research Centres, Agrochemical Product Development, Geneva, Switzerland. The analytical work was carried out in September 1996.

The highest average indoxacarb residues occurred immediately after the last of six applications (0 DALA), corresponding to 0.21 µg/cm^2 at Germany and 0.22 µg/cm^2 at Italy, and declined to 0.03 and 0.02 µg/cm^2 respectively, at these sites by 28 days after the last application. Residues were shown to decline with DT_{50} values of 11 days (Germany) and 9 days (Italy). DT_{90} values were 38 days (Germany) and 31 days (Italy).

Recovery values for lab control fortifications ran concurrently with the treated samples in all the trials, were within 79 to 107% (n = 8). All of the recoveries were within 70 to 110%, and the relative standard deviations were less than or equal to 20% for all trials; therefore, the analytical methods used performed well for the determination of indoxacarb in dislodging solution.

III. CONCLUSION

This study describes the dislodgeable foliar residue behaviour of indoxacarb from apple leaves in one season of study in the Europe for application and sampling conducted according to GAP. The residue data show that the highest average residue at a site was 0.22 µg/cm^2 and residues declined on apple leaves with time with an average half-life of 10 days.

(Guinivan, R.A., Enriquez, M.A., Schelling-Schiavo, M., 1997)